The American Journal of Medical Technology

NOVEMBER-DECEMBER, 1954

Vol. 20, No. 6

OFFICIAL PUBLICATION

Published Bi-Monthly by The American Society of Medical Technologists
Printed by The Gulf Printing Company
Business and Editorial Office: Suite 25, Hermann Professional Bldg., Houston 25, Texas
Printing Office: 3301 Buffalo Drive, Houston 6, Texas

THE

AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

CONTENTS

VOLUME TWENTY NUMBER 6

NOVEMBER-DECEMBER

1954

Page

The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

American Society of Medical Technologists

SOCIETIES CHARTERED BY THE A. S. M. T.

1. **Texas Society of Medical Technologists**
2. **Kentucky State Society of Medical Technologists**
3. **Nebraska Society of Medical Technologists**
4. **Minnesota Society of Medical Technologists**
5. **Oklahoma Society of Medical Technologists**
6. **Colorado State Society of Medical Technologists**
7. **Indiana Society of Medical Technologists and Laboratory Technicians**
8. **Illinois Medical Technologists' Association**
9. **District of Columbia Society of Medical Technologists**
10. **Pennsylvania Society of Medical Technologists and Laboratory Technicians**
11. **Georgia Society of Medical Technologists**
12. **Wisconsin Association of Medical Technologists**
13. **Ohio Society of Medical Technologists**
14. **Empire State (N. Y.) Association of Medical Technologists**
15. **Arkansas Society of Medical Technologists**
16. **Louisiana State Society of Medical Technologists**
17. **Tennessee Association of Medical Technologists**
18. **Massachusetts Association of Medical Technologists**
19. **Michigan Society of Medical Technologists**
20. **New Hampshire Society of Medical Technologists**
21. **California Society of Medical Technologists**
22. **Virginia Society of Medical Technologists**
23. **Association of Oregon Medical Technologists**
24. **Utah Society of Medical Technologists**
25. **Mississippi State Society of Medical Technologists**
26. **Maryland Society of Medical Technologists**
27. **Missouri Society of Medical Technologists**
28. **Kansas Society of Medical Technologists**
29. **North Carolina Society of Medical Technologists**
30. **Delaware Society of Medical Technologists**
31. **Connecticut Society of Medical Technologists**
32. **New Mexico Society of Medical Technologists**
33. **South Dakota Society of Medical Technologists**
34. **North Dakota Society of Medical Technologists**
35. **Florida Division of A.S.M.T.**
36. **New Jersey Society of Medical Technologists**
37. **Washington State Society of Medical Technologists**
38. **West Virginia State Society of Medical Technologists**
39. **Wyoming Society of Medical Technologists**
40. **Alabama Society of Medical Technologists**
41. **Montana Society of Medical Technologists**
42. **Vermont Society of Medical Technologists**
43. **Idaho Society of Medical Technologists**
44. **Iowa Society of Medical Technologists**
45. **Hawaii Society of Medical Technologists**
46. **Nevada Society of Medical Technologists**
47. **Rhode Island Society of Medical Technologistc**
48. **South Carolina Society of Medical Technologists and Laboratory Technicians**
49. **Maine Association of Medical Technologists**
50. **Arizona Society of Medical Technologists**

TO CONTRIBUTORS AND CORRESPONDENTS OF THE JOURNAL

Published January, March, May, July, September, November

Editorial and Business Office: Suite 25, Hermann Professional Bldg., Houston 25, Texas

ORIGINAL ARTICLES

Brief reports of new and interesting methods and results pertaining to the laboratory field are solicited. None will be considered for publication except with the understanding that it becomes the property of the Society.

Manuscripts must be typewritten and double spaced. (Do not submit carbon copies.) All figures and tables must be in form for the printer.

ILLUSTRATIONS

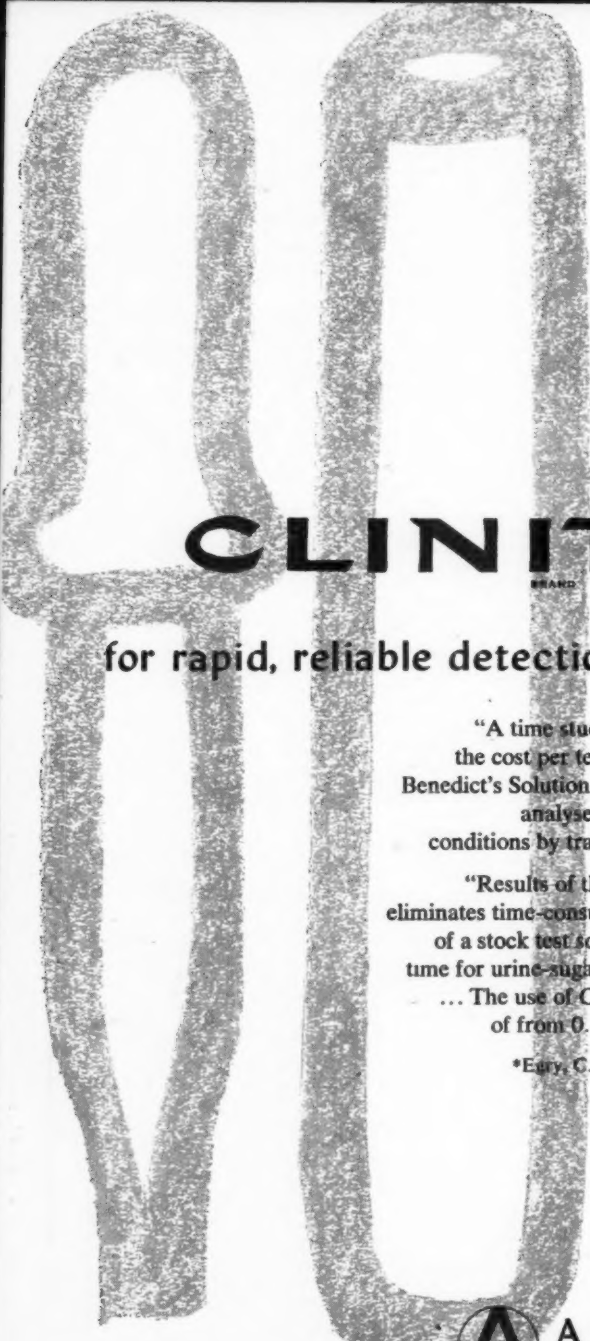
Cuts must be supplied or can be arranged for by the Editor. Expenses defrayed by the author if in excess of one cut. Drawings must be on white paper in India ink.

Reprints of original articles can be obtained. Order blank with price list is sent with galleys.

SUBSCRIPTION PRICE

$4.00 a year in United States, Canada, and Countries within the postal union; $4.50 for countries outside the postal union; single copy, $0.75. The subscription of members of the American Society of Medical Technologists are included in the membership dues and are handled by the Executive Secretary of the Society. Other subscriptions should also be sent to the Executive Secretary, Suite 25, Hermann Professional Bldg., Houston 25, Texas.

Please notify Business Office of change of address



saves time
and
money

CLINITEST®
BRAND

for rapid, reliable detection of urine-sugar

"A time study was undertaken to determine the cost per test for urine-sugar analysis using Benedict's Solution and CLINITEST. Urine-sugar analyses were performed under normal conditions by trained personnel in two hospitals.

"Results of the tests show that CLINITEST eliminates time-consuming preparation and handling of a stock test solution, and reduces the running time for urine-sugar analysis by about 50 per cent. ... The use of CLINITEST resulted in a saving of from 0.18 cent to 1.23 cents per test."*

*Egry, C. R.: Hosp. Topics *32*:31 (July) 1954.

AMES DIAGNOSTICS

Adjuncts in Clinical Managem

AMES
AMES COMPANY, INC. | ELKHART INDIANA
Ames Company of Canada, Ltd., Toron

53454

Please Mention Publication When Writing Advertisers

The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 20 NOVEMBER-DECEMBER, 1954 NUMBER 6

ANTISTREPTOLYSIN-O TITRATION—A DIAGNOSTIC TOOL IN RHEUMATIC FEVER*

MIRDZA L. A. LABSVIRS, B.A. M.T. (ASCP)
St. Luke's Hospital, Duluth, Minnesota

I. **Introduction.**—Review of the Literature.

Rheumatic fever is strictly a bacterial infection. It is caused by Group A streptococci, which is known to produce a clear zone of hemolysis on blood agar, or are beta-hemolytic. There are some exceptions which will be pointed out later. As is also known Group A streptococci are responsible for nearly all streptococcic infections in human beings.

In 1932 Todd reported that Group A streptococci form at least two hemolysins demonstrable in vitro. They are:

(1) "Streptolysin S" which is formed in vitro in the presence of serum. This is very toxic, producing beta hemolysis on blood agar plates, or death of the experimental animal. Its corresponding antibody in living tissue has not been investigated as yet, and

(2) "Streptolysin O" which is produced in vitro in serum-free broth and is heat and oxygen labile. In the reduced form this specific hemolysin is lytic to erythrocytes of several mammalian species and is also leucocidic. It has not been demonstrated as being *erythrogenic*.

This Streptolysin-O, it is thought, is released by Group A streptococci during the manifestation of disease, but it is not known to be responsible for all of the streptococcal pathology. When introduced into living tissue this Streptolysin-O will cause the production of antibodies which have been called Anti-strepotolysins-O. These are associated with the gamma globulin of plasma.

Many studies have been done to demonstrate the concentration of this Antistreptolysin-O in serum from patients having, or having had in the recent past, a Group A streptococcal infection. They have been found in the serum in 90 per cent of the individuals suffering of or recovering from Group A streptococcal infection. They have also been demonstrated in the fetal blood in amounts that are often greater than those in maternal blood.

* Third ASMT award. Read before Convention, June, 1954. Miami Beach, Florida.

In 1932 Todd worked out a satisfactory laboratory test for determining the presence of these antibodies and for measuring their concentration in serum. Since that time several other investigators (Rantz, Randall, Hodge, Swift) using Todd's method, at least in principle, have studied innumerous cases of Group A streptococcal infections. It is the pooled findings of Todd, these, and other investigators that have made it possible to give the table of values found later in this article.

The Todd test is a lysis test, utilizing constant amounts of antigenic Streptolysin-O, varying dilutions of homologous immune sera, and constant amounts of 5 per cent washed rabbit red blood cells suspended in a buffered diluent. Virtually it is a titration test and the titre is expressed in Todd units, (defined below) which value is referred to as the "Antistreptolysin-O titre" or the "AST-O" for short. The principle involved in the test is apparently immunological specificity of the first order so that both or only the last of the following phenomena will occur, namely

(1) A combination of antigen and antibody, immunologically neutralizing each other. This reaction is specific so that in the presence of red blood cells no hemolysis occurs, and/or

(2) A combination of antigen and red blood cells provoking hemolysis of the cells. This reaction is non-specific and occurs when the antibodies are absent or the concentration of antibodies exceeds the maximum.

As stated later the serum is inactivated for ½ hour at 56° C. The serum having been inactivated thus the complement is destroyed, and since no fresh serum is added the lysis cannot be a complement-fixation phenomenon but a toxic injury of the red blood cells by the Streptolysin-O antigen as evidenced by the hemolysis. The end point of the test is indicated by hemolysis and that serum dilution containing the least amount of serum showing no hemolysis is the end point.

Todd standardized Streptolysin-O, defining a unit as that 0.5 ml of concentrate of toxic broth filtrate from an 18-hour Group A streptococci culture which will completely hemolyze 0.5 ml of a 5 per cent suspension of washed rabbit blood cells when incubated at 37° C for 1 hour. This Todd unit is also referred to as the "minimum hemolytic dose," the "M.H.D." Todd also standardized the corresponding antibodies defining one unit of Antistreptolysin-O as the amount of serum required to neutralize two and one-half minimum hemolytic doses of Streptolysin-O.

Todd showed that individuals considered normal otherwise may have a titre of 50-100 Todd units. This may be explained away presumably by regarding these individuals as having been

occultly immunized by Group A streptococci. All values above the 50-100 unit range, Todd considered abnormal and diagnostic; relatively high AST-O in human sera being consistently associated with Group A streptococcic infection. Todd (Randall, Rantz, Coburn, Paul and others) found this to be true of the approximately 90 per cent of the patients suffering from Group A streptococcic infections. However Todd has shown that there may be certain strains of streptococci which do not elaborate this specific Streptolysin-O and therefore these patients will have no serum AST-O. Todd pointed out that among the Group A streptococcic infections high AST's-O are present especially in active cases of Rheumatic Fever and Glomerulo-nephritis. While titres may rise to 5000 units and above, the literature also reports abnormal titres much lower than this, some being only slightly above the maximum "normal" range. Instances of this are the titres of 125 units found in the 0.2 per cent of children having acute Rheumatic Fever as studied in England. This phenomenon may persist throughout the course of the disease and may be explained either by the absorption of Streptolysin-O by the tissues, or by the difference in antigenic or hemolytic properties of streptococci belonging to Group A. Later investigators (Harris, Harris) proved that a similar "low" serum AST-O may be found in patients suffering from a renal disease associated with a nephrotic syndrome. In these instances urinary proteins were shown to contain larger quantities of the Antistreptolysin-O than serum.

Other studies have reported findings on other types of Group A streptococcic infections. A composite of the abnormal titres reported in the literature is the following table.

TABLE I
AST-O Titres in Disease

DISEASE	AST-O
Rheumatic fever (active)	2500 and above
Rheumatic fever (recurrent)	100 or slightly above
Acute glomerulonephritis	Up to 2500 or above
Disseminated lupus erythematosus	12-250
Periarthritis nodosa	12-835
Collagen diseases	50-250
Erythema nodosum	125-1250
Henoch-Schoenlein purpura	33-250
Rheumatoid arthritis (early)	"Low"
Rheumatoid arthritis (advanced)	"High"

II. The Research Problem, Statement of

My 15-month research problem began in December 1952 with a view of finding how useful the AST-O might be as a diagnostic tool in Group A streptococcic infections and particularly in Rheumatic Fever when in the patients the symptoms were obscure. I first applied the AST-O test to clinically diagnosed patients. Then I became interested in patients without clinical

symptoms other than recurrent sore throat, or frequent nose bleeds, or high sedimentation rates. For a short interval I included patients clinically diagnosed as scarlet fever and virus pneumonia patients. These two latter types of patients gave findings that were definitely without value and I dropped them from the study. In all the other cases an established routine of making throat cultures and doing AST-O tests periodically was established. The nose and throat cultures were made simultaneously with the first serum determination for the AST-O, doing this before antibiotic therapy had been instituted. I cultured the nose and throat specimens on blood agar plates, inoculating the surface with a loop by the streak method and culturing anaerobically in a 10 per cent carbon dioxide atmosphere at 37° C. overnight. Later I found that by culturing the inoculum in fluid thioglycollate medium overnight and then subculturing by the streak method on blood agar plates the material could be incubated aerobically and the same results obained. If the characteristic beta-hemolytic colonies were found, Group A specificity was determined by the Lancefield precipitin method as given below.

The organisms were removed from the plate, suspended in 25 ml sterile brain heart infusion broth in a 50 ml conical centrifuge tube under sterile conditions, incubated for 18 hours at 37° C aerobically, and centrifuged for 15 minutes at 2000 rpm. The supernatant fluid was discarded and 2.5 ml of 20/N HCl added to the precipitate in the bottom of the cone, and this boiled in a water bath for 10 minutes. This material was recentrifuged at the same speed for another fifteen minutes and the supernatant fluid pipetted to a small test tube. This fluid was treated with 2 drops of N/1 NaOH. Then 0.05 ml of this fluid was transferred by means of capillary attraction from a 0.2 ml pipette to a capillary tube, 80 millimeters long and 7 millimeters diameter, one end being sealed. By the same capillary method this 0.05 ml of fluid was overlaid with an equal volume of Group A streptococci diagnostic sera (Purchased from Lederle). The loaded capillary was mounted carefully in a suitable rack and left undisturbed for 30 minutes at which time it was read for turbidity at the juncture of the two fluids. The presence of a white "ring" indicated a positive test. Simultaneously a control substituting normal saline for the HCl-NaOH digest was set up. If by this method the throat or nose culture showed Group A streptococci the patient was retained in my study. At stated intervals erythocyte sedimentation rates were determined and white blood cell counts made.

In addition to the

(1) septic sore throat, positive for Group A streptococci

(2) abnormal sedimentation rates, and

(3) white blood cell count, all the following symptoms were noted in the patient as well:
(4) recurrent nose bleeds, and
(5) low grade fever.

Of the total number of 860 titres representing random tests and tests done in serial on patients I wish to include only in this paper a discussion of those on whom I have done serial tests.

The patients, subjects of my research, were hospitalized in three separate hospitals. All testing was done, however, in this institution, and either the patients or the specimens were sent to this research laboratory where all the tests including the AST-O were made and data assembled. The AST-O was made bi-weekly or every three weeks during the acute stage, and every 4-5 weeks during convalescence for 3 to 4 additional months. In instances where AST-O values were abnormal the attending clinician instituted chemotherapy.

Even after convalescence some of the test subjects of this research are being maintained prophylactically on a periodical injection of penicillin, erythromycin or such other antibiotics as determined by sensitivity tests in a great number of cases.

Patients once infected with Group A streptococci, it is thought, become very susceptible to recurrent infections depending on such environment changes as temperature, cleanliness, and kindred factors. Irrespective of the level to which the titre drops therapeutically, a cold or another slight provocation may cause a rise in the titre, and for these reasons the test subjects under my observation returned for "checkups." The "check-up" data, especially the AST-O, have been included in the table found below together with data from the acute and convalescent stages.

III. Experimentation on the Problem

a. The Reagents

1. *Antigen.* Streptolysin-O reagent. (Previously purchased from Cutter, Berkeley, California, but now from Difco, Detroit, Michigan.) This vital component is a dehydrated solid obtained from the broth filtrate of an 18-hour culture of Group A streptococci. This is compounded with the amino acid, cysteine, and an alkaline buffer salt, which mixture is packaged in different sized vials and the solid dispensed by volume, not by weight. The smallest volume on the market is 10 ml and the largest 25 ml.

To reconstitute the antigen, approximately an equal volume of distilled water is added. For instance to a 10 ml vial, 10 ml of distilled water are added. This is mixed well to dissolve and is used immediately since it remains stable but 2 hours. 0.5 ml of this reconstituted Streptolysin-O reagent is so standardized

by the manufacturer that it will contain one combining unit of Streptolysin-O.

2. *Diluent.* This is an approximately isotonic solution of a buffer whose pH range is between 6.5 and 6.7. The buffer salts vary with the commercial houses producing them but differ little from, if they are not identical to the following:

Phosphate-Buffer-Saline Salt Solution

NaCl	4.2 gms.
KH_2PO_4	3.17 gms.
$Na_2HPO_4.12\ H_2O$	3.59 gms.
Distilled water	1000.0 ml

Since the pH range of the buffer adapted to the test must be pH 6.5-6.7, I check it with methyl red indicator periodically, if a potentiometer is not available. The solution keeps approximately two weeks without readjustment but it must be stored in the interim at about 4° C. If a liter is made at a time it will take care of approximately 40 tests.

3. *5 Per Cent Rabbit Blood Cell Suspension.* Rabbit blood was obtained by cardiac puncture and mechanically defibrinated by shaking with glass beads for at least 5 minutes. The defibrinated blood was placed in 15 ml conical centrifuge tubes and the red blood cells washed with normal saline until free of red tinge in the supernatant fluid. This usually required three washings—each being accomplished by 5 minutes of centrifuging at 2000 rpm. Obviously 5 ml of the packed red blood cells were suspended, this time in the isotonic buffer solution described above and the volume made up to 100 ml with the same solution. For 25 tests, 200 ml are ample but this suspension must be made fresh at the time of the test. Human Group O red blood cells, obtained from the blood bank, were used initially but found less sensitive than rabbit blood cells and their use was discontinued.

b. **Outline of Technique.** I followed the technique of Rantz and Randall for determining the AST-O, but I used 14 tubes for the test instead of 12 tubes besides the 2 controls. The tubes are Pyrex glass Wassermann size. I use the regular agglutination rack to support them. All glassware used in the test proper and for diluting sera must be chemically clean and sterile.

1. *Serum dilutions.* The serum is inactivated at 56° C. for 30 minutes to destroy the complement. The following dilutions are made then in sterile 18 x 150 mm test tubes:

1:10 —0.5 ml of serum and 4.5 ml of diluent.
1:100—1.0 ml of 1:10 dilution and 9 ml diluent.
1:500—2.0 ml of 1:100 dilution and 8 ml diluent.

To avoid any false AST-O tests, the sera must be removed from the cells immediately and stored at 4° C until it is to be used. It should be free from hemolysis and from other contaminations.

2. *The Technique Proper.* The tubes are placed in a Wassermann rack or agglutination rack and numbered from left to right 1 to 14 inclusively. The above dilutions are pipetted into the sterile tubes according to this outline and the procedure followed as indicated:

	1:10		1:100					1:500						*
Tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ml. dil. serum	0.8	0.2	1.0	0.8	0.6	0.4	0.3	1.0	0.8	0.6	0.4	0.3	0.2	0.1
Ml. Buffer sol.	0.2	0.8	0.0	0.2	0.4	0.6	0.7	0.0	0.2	0.4	0.6	0.7	0.8	0.9
	Shake Gently to Mix													
M. Streptolysin-O	Add 0.5 ml. to tubes 1-14 inclusively. Shake gently to mix. Incubate at 37° C for 15 min.													
Ml. 5% Rbc susp.	Add 0.5 ml. to all tubes including the two controls—(Tubes 15 and 16). Shake gently to mix. Incubate at 37° C for 45 min. Shake gently to resuspend the cells at the end of the first 15 min. Centrifuge tubes after 45 min. incubation for 1 min, at 1500 rpm. Read highest dilution showing no hemolysis.													
Unit Value in Tube	12	50	100	125	166	250	333	500	625	833	1250	1665	2500	5000

3. *Converting to Todd Units.* The following scheme will help calculate the units mentioned in the outline above. Since Todd defines one unit (U) Streptolysin-O suspension as 0.5 ml of a suspension which will hemolyze 0.5 ml of 5 per cent rabbit red blood cell suspension when incubated at 37° C for 1 hour, Tube 1, for instance has 12 Todd units. This is the manner in which that value was obtained:

In Tube 1, according to the outline, 0.8 ml of 1:10 dilution of the serum was used which means that virtually that tube contains but 0.08 ml of undiluted serum. But the 0.08 ml is sufficient to "neutralize" the 0.5 of Streptolysin-O or the unit (U) of antigen, leaving intact the required amount according to Todd or 0.5 ml of the red blood cell suspension. Hence this 0.08 ml serum contains 1 antistreptolysin O Todd unit (A.U.). Thus

If 0.08 ml or 8/100 ml serum contains 1 Todd unit

Then 1/100 ml contains 1/8 of 1 Todd unit and the undiluted serum or

And 100/100, contains 100 x 1/8 or 100/8 or 12.5 units.

---

* Controls are two-fold-*Cell control.* This is the 15th tube in the rack and is set up by using 1.5 ml. of the diluent and 0.5 ml. of the 5 per cent red blood cell suspension. This tube should show no hemolysis and is used to determine the slightest amount of. hemolysis in the other tubes. *Lysin control.* This is the 16th tube in the rack and is set up by using 1.0 ml of diluent, 0.5 ml of Streptolysin-O, and 0.5 ml of red blood cell suspension. This tube should show marked hemolysis.

The end of the test is to determine that dilution of serum that contains enough antibodies (AS-O), to keep the antigen (S-O), from hemolyzing even the slightest number of red blood cells. In other words the tube of the serum dilution remaining colorless, i.e., free of any tinge of laked blood, is the end point, and is evaluated in terms of Todd units as discussed.

The fraction is disregarded and the tube is said to contain 12 Todd units of Antistreptolysin-O per ml serum. Obviously the number of units or the titre in any given tube is really the reciprocal of the serum dilution of that tube and is in other words the number of units of AS-O that are found per ml serum.*

IV. **Test-Subjects and Data.**

Although I worked with 393 patients I wish to detail the data on only 93 on whom serial tests were made. Eighty of these were children between ages of 4 and 14 years and are tabulated as one group. Two children ages 8 and 12 years, whom I consider exceptions, are tabulated as a second group. Four patients considered as exceptions are tabulated as the third group. One adult, age 56 years, is tabulated alone for reasons I shall give later. And, lastly, 6 other cases are tabulated separately as convalescents having had a history of Rheumatic Fever but at the time of my testing were experiencing only recurrent sore throats.

The 80 children in my first group were hospital patients but there were not enough clinical findings to warrant a diagnosis. For about three weeks they had a "cold," low grade fever accompanied by frequent tonsilitis, and nose bleeds. The next several weeks the clinical symptons changed but as I indicate below the Antistreptolysins-O multiplied rapidly in the blood stream and my AST-O readings mounted dramatically.

The two children, 8 and 12 years of age, I considered exceptions because there was no Antistreptolysin-O titre in their blood during the first three weeks, and this I would have expected for the same was true of the 80 children mentioned above, but by the end of the sixth week, when the throat cultures still remained positive for Group A streptococci, the titre only reached a reading of 125 Todd units and persisted at that level to the end. Clinical symptoms of Rheumatic Fever were demonstrated at this time. These children perhaps belong to that 0.2 per cent group mentioned in the literature to which I referred in the earlier part of this paper.

The 4 cases ranging between 25 and 35 years of age I considered exceptions because the sedimentation rates did not correlate with the AST-O. There were no clinical symptoms except one patient had a swollen index finger of the right hand and the other a swollen toe. Both felt fatigued at all times. The sedimentation rates were consistently normal in three patients while the AST-O reached 625 Todd units and throat cultures demonstrated the presence of Group A streptococci in 40 per cent of the de-

* Todd defines 1 Antistreptolysin unit (AU) as the ml of immune serum necessary to neutralize (I think "render inactive" the better interpretation) 2.5 U or 2.5 MHD of streptolysin-O. Letting U or MHD represent one Unit of antigen and A.U. one unit of antibody, the following holds: 1 A. U. = 2.5 U. In the test this interpretation is seemingly disregarded.

terminations I made on them. When the sedimentation rate on the fourth patient reached, for instance, 17 millimeters/1 hr(W), the AST-O read 1250 Todd units.

The individual case was a woman aged 56 years who became suddenly very ill, complaining of nausea, vomiting and chills. Three weeks after the initial infection her AST-O reached 250 Todd units. All throat cultures made on her were positive for Group A streptococci and in spite of antibiotics and cortone therapy 97 per cent of the cultures of her throat remained positive during the ten months the case was under study. There was a great difference in her AST-O between the third and sixth weeks of infection when compared to the titres displayed by the children in the 4-14 year age span. While the children's range was from 50 to 833 Todd units, the titre of the 56 year old patient ranged from 250 to 1665 Todd units. After six weeks of infection her titre climbed to 2500 and then on up to more than 5000 Todd units. The children's range spanned between 500 and 5000 and above. In other words the lower end of the range attained by the children in the sixth week was much lower than that of the adult although both the lady and the children ultimately mounted to the same 5000 height and above. Apparently the children's tissues were able to release antibodies much more rapidly once they began to do so than were the adult's.

The 6 convalescents including a 17-year old boy and 5 others ranging from 30 to 35 years of age demonstrated high titres at the onset of reinfection. These were never a part of the study in their active stages. The 17-year old patient, for instance, had an AST-O reading as high as 2500 Todd units. He was suffering from a sore throat, fatigue and dizziness. Chemotherapy was instituted when the doctor first saw him and later a tonsillectomy

TABLE II
Research Data on 93 Rheumatic Fever Patients

No. Cases	Age in Years	1—3 Weeks Post Infection		3—6 Weeks Post Infection		6 Weeks and Later Post Infection	
		AST-O	Throat Culture	AST-O	Throat Culture	AST-O	Throat Culture
80	4-14	0-50	Positive 97%	50-833	Positive 40-50%	500-5000	Positive 40-50%
2	8-12	0	Positive 98%	100-125	Positive 95%	125	Positive 95%
4	25-35	0-12	Positive 95%	166-625	Positive 40%	Not followed up	
1	56	250	Positive 100%	250-1665	Positive 97%	2500-5000	Positive 97%

No. Cases	Age in Years	CONVALESCENT STAGE	
		AST-O	Throat Culture
5	30-35	166-250	Positive at the time of reinfection.
1	17	50	Negative

was performed after which his titre dropped to the low reading of 50 Todd units as tabulated in Table II. The older members of this group had similar high readings initially but upon chemotherapy these dropped to the low range of 166-250.

(Wherever clinical data are used I embodied them by authorization of Dr. A. H. Wells, pathologist of my hospital, for which privilege due acknowledgment is herewith made).

Table II if properly interpreted indicates that there are three separate phases in active Rheumatic Fever. These phases are the following:

1. *Phase I* which covers a period of 1-3 weeks after infection with Group A streptococci. There is hardly any rise in AST-O. Clinically there are a low grade fever, a simple tonsillitis, frequent nose bleeds, an elevated sedimentation rate, and a slightly elevated white blood cell count. The only common significant and somewhat diagnostic feature is the throat culture demonstrating the presence of beta-hemolytic streptococci belonging to Group A, as established by the precipitin test.

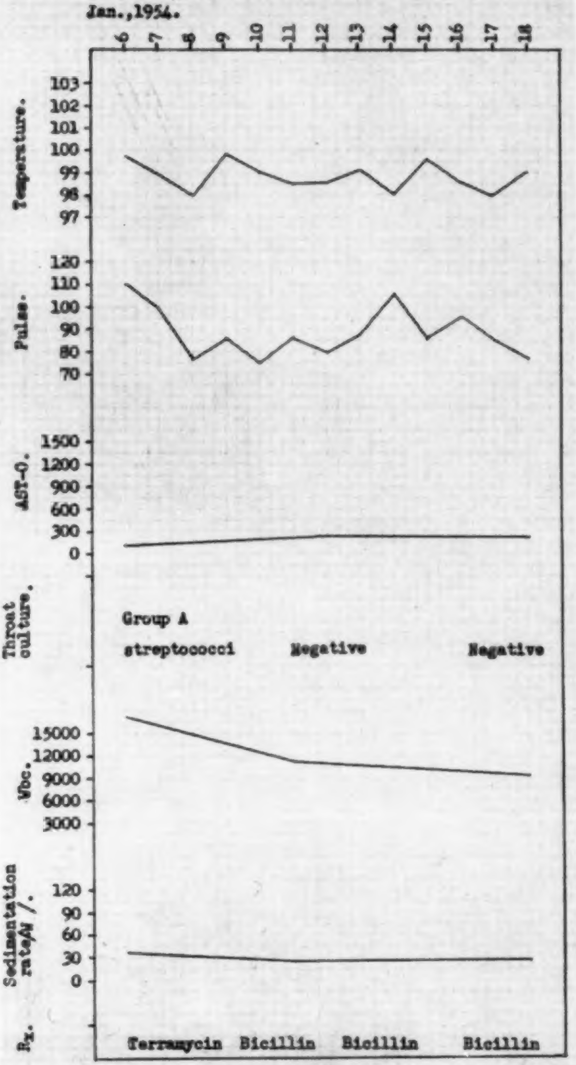
2. *Phase II* which covers a period of 3-6 weeks after infection. There is a rise in the AST-O reaching a maximum of 833-1250 Todd units. The clinical findings are the same as for Phase I, i.e., an increase in the sedimentation rate and in the white blood cell count. No involvement of the heart is noticed. About 40-50 per cent of the throat cultures demonstrate Group A streptococci. According to the literature and also confirmed by my findings negative throat cultures do not mean that the organisms have disappeared completely from the throat but that they have become active in paranasal sinuses behind the throat membranes, and in the lymph nodes. I also found that during the period of chemotherapy, Group A beta-hemolytic strains of streptococci became gamma-hemolytic and lost their specificity resulting in a negative precipitin test for Group A streptococci.

3. *Phase III* which covers a period following the initial 6 weeks and usually demonstrates an AST-O that reaches 5000 or more Todd units. The sedimentation rate is high in most cases. The throat cultures are positive in 40-50 per cent of the cases. In this phase clinical symptoms such as involvement of the heart are demonstrable.

The following four graphs are presented in order to show a correlation of laboratory data with the clinical findings of typical cases.

Graph I is a typical graph in the first phase of a patient of the 4-14 year group. The graph is that of a 6-year old male who began to complain of being very weak and who tired easily. This graph demonstrates irregular pulse and fever curves, sedimentation rates ranging from 33 to 25 mm/hr (W), white blood cell counts from 17,200-9,050 cells/cu mm and an AST-O reaching

Graph I. Typical First Phase Study.



166 Todd units. The throat culture was positive for Group A streptococci at the time of admission, and became negative after the chemotherapy was started. The patient had no complaints of pain or other specific discomfort. The heart demonstrated a normal rate and rhythm, no audible murmurs being heard. The tonsils showed a few patches of exudate in the crypts, but during the study these diseased tonsils were removed.

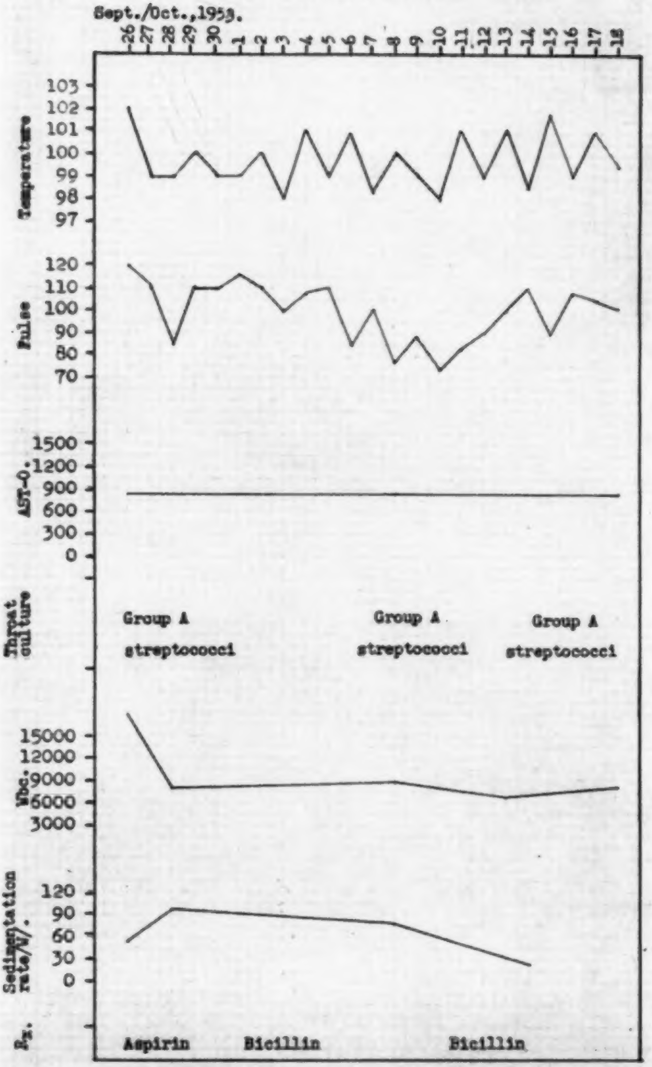
The above symptoms also applied to the other patients studied in this group in the first phase except that a great number of them suffered in addition recurrent nose bleeds and slight muscle tenderness. These latter details are mentioned, although not graphed, to make the picture complete.

Graph II is a typical graph in the second phase of a patient in the 4-14 year age span. This graph demonstrates an increased irregularity in pulse and fever, a sedimentation rate ranging from 93-44 mm/hr (W), a white blood cell count ranging from 16,000-5,200 per cu mm and an AST-O of 625 Todd units. The throat culture at the time of admission demonstrated Group A streptococci and became negative after chemotherapy was started. The patient concerned, a 7-year old male, was admitted to the hospital, demonstrating pain in the joints, especially in the left elbow, and also had a body rash. He had had a sore throat for about two weeks previously. He seemed to suffer much muscularly, finding it painful even to have the tourniquet applied. His heart was normal.

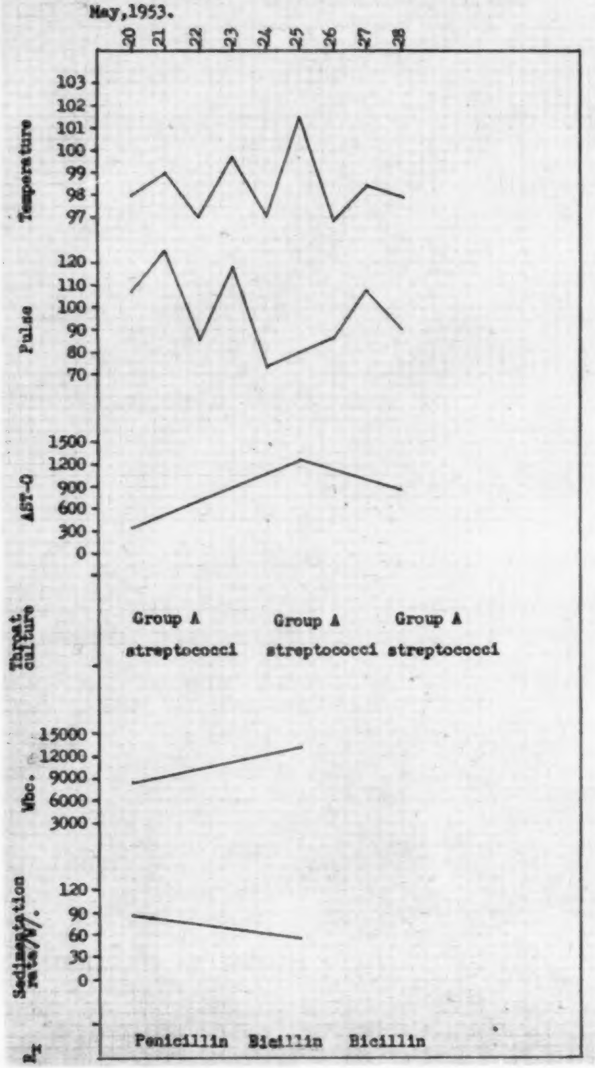
These findings were common to the other patients belonging to this group and in this phase.

Graph III is a typical graph of a patient in the third phase, and represents a patient of the 4-14 year span. This graph demonstrates a sedimentation rate ranging from 80-55 mm/hr (W), a white blood cell count from 8,600 to 11,500 cu mm and an AST-O reaching a peak of 1225 units the fifth day after admission. The patient concerned was a 6-year old male who demonstrated a slight cardiac enlargement, a development of systolic apical murmur, felt to be on the basis of mitral valvulitis of relative mitral insufficiency according to the findings of the attending physician. The positive Group A streptococcic throat was further complicated by chronic tonsilitis and adenoiditis. Group A streptococci were demonstrated in the throat in every instance during the study until post-tonsillectomy when the first negative culture was obtained. There was a slight disappearance of serum antibodies after eight days hospitalization. This may be explained either as a result of chemotherapy or absorption by the tissues. Might it also be that in spite of the bed rest during hospitalization the streptococci remained active behind the membranes and paranasal sinuses releasing Streptolysin-O even in the third phase of Rheumatic Fever? This patient made a progressively slow re-

Graph II. Typical Second Phase Study.



Graph III. Typical Third Phase Study.



covery and within approximately 6 months after tonsillectomy he was considered as being normal.

Graph IV is that of a 56-year old female. This graph excludes the temperature and pulse curves, and the clinical findings, but includes a new feature, namely antibiotic and hormone therapy. The graph emphasizes that while the patient was on bed rest, her AST-O fluctuated greatly. The sedimentation rates were low at the time when the titre curve reached high peaks and the white blood cell count hovered about a plateau at the 12,000/cu mm level.

As soon as ACTH therapy was started the AST-O titre dropped 1041 units per day but the throat culture remained positive to Group A streptococci. When magnamycin therapy was instituted the throat culture became negative in a few days and then positive again raising the question of drugfastness on the part of streptococci toward magnamycin. In vitro this drugfastness was confirmed by a sensitivity test. Chemotherapy was continued, but the nature of the antibotics varied so that the throat became negative to Group A streptococci and by the end of 4 months the AST-O dropped to as low a level as 125 Todd units.

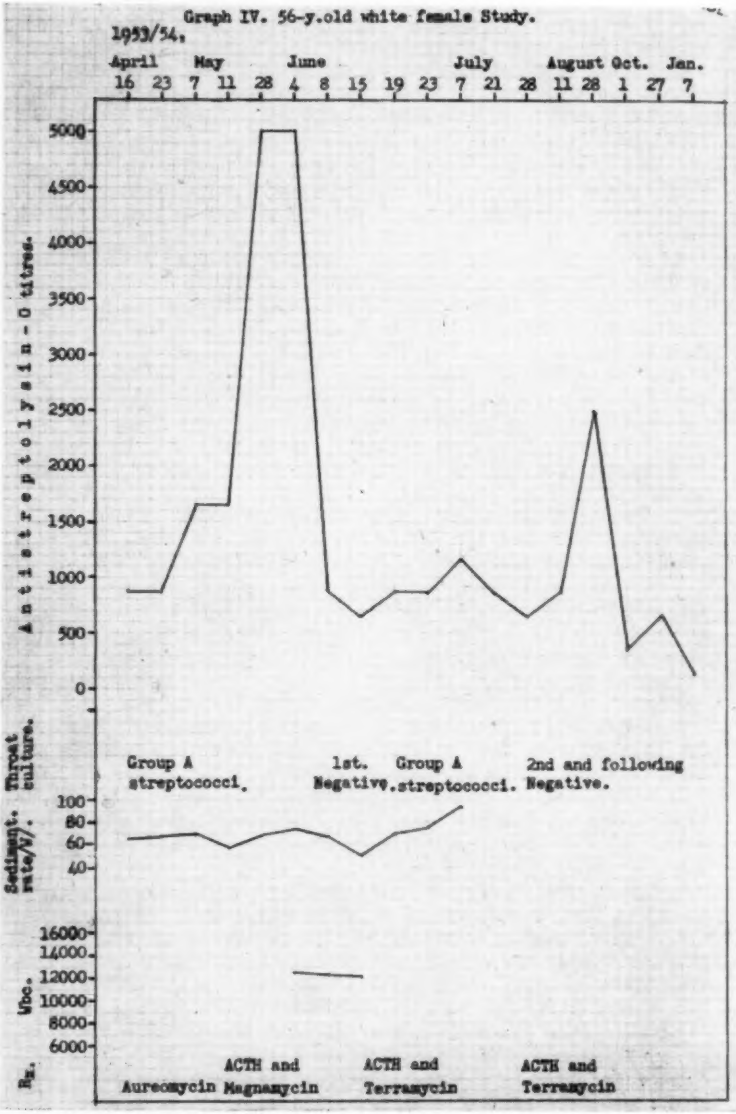
In this as in the other graphs the discrepancy between sedimentation rate and the AST-O is evident, but the AST-O is more consistent with the real gravity of the disease.

V. *Conclusion.* From the foregoing it is apparent that the AST-O is a diagnostic tool in Rheumatic Fever and that as a tool it is a more sensitive indicator of the gravity of the pathology and the patient's response to therapy than is any other laboratory means of diagnosis.

Pertaining to titres it would appear that

1. 12-100 Todd units may be considered the normal limits if the titre remains fixed at that level and the throat cultures remain negative to Group A streptococci.
2. A low AST-O (50 Todd units) is more diagnostic than a high titre in the initial stages of Rheumatic Fever. Together with a positive throat culture it indicates the first phase of Rheumatic Fever.
3. A titre of 50-833 Todd units with a positive throat indicates an active case or the second phase of Rheumatic Fever.
4. A titre ranging from 500-5000 or above indicates the third phase of Rheumatic Fever and is usually associated with clinical symptoms enabling an immediate diagnosis.
5. A drop in AST-O titre during the treatment means recovery.

Pertaining to titres my research raises the question, do antibodies appear and disappear more readily in youth? The 80 children between ages 4 and 14 years cited above seem to answer in the affirmative for the rapid appearance of antibodies, and the


Graph IV. 56-y.old white female Study.
1953/54.
April May June July August Oct. Jan.
16 23 7 11 28 4 8 15 19 23 7 21 28 11 28 1 27 7
Antistreptolysin - O titres.
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Throat culture.
Group A streptococci.
1st. Negative.
Group A streptococci.
2nd and following Negative.
Sediment. rate/V/.
100
80
60
40
Wbc.
16000
14000
12000
10000
8000
6000
Rx.
Aureomycin
ACTH and Magnamycin
ACTH and Terramycin
ACTH and Terramycin

17-year old in the affirmative for rapid disappearance of antibodies.

Pertaining to techniques my research indicates that the following points are pertinent

1. A throat culture should be obtained at the same time as the blood sample is drawn and the grouping of the streptococci determined by the Lancefield precipitin test method. Serum should be removed from the cells as soon as the blood is clotted and stored at 4° C until ready to use.

2. To avoid a false negative throat culture the specimens should be obtained before chemotherapy is instituted or 72 hours after the antibiotic is discontinued.

3. To avoid the inadequacy of a random AST-O, repeated determinations should be made periodically during and after infection at biweekly intervals over a period of 4 to 6 weeks following infection. In other words the AST-O determination should begin in the acute phase, follow through illness, and project into convalescence. Note. In this research only those patients have been included who were Rheumatic Fever suspects, or whose diagnosis had been made clinically. The high values associated with Glomerulonephritis have not been treated in this paper, and are excluded from Rheumatic Fever data.

VI. *Summary*. A review of literature on Streptolysin-O has been given and research involving particularly the AST-O on 93 out of 393 patients reported. The records indicate that the AST-O is a diagnostic tool in Rheumatic Fever and superior to any other laboratory means of diagnosis.

VII. *Acknowledgment*. Due acknowledgment is made to my pathologist, Dr. A. H. Wells, for permission to perform this piece of research; to Sr. M. Alcuin, OSB, my former teacher for timely criticism and advice in the preparation of this paper; to attending pediatricians for their splendid help in the many details of this study; and, to the student medical technologists under my supervision who aided me technically.

BIBLIOGRAPHY

1. Todd, E. W., "Antihaemolysin titres in haemolytic streptococcal infections and their significance in rheumatic fever." British Journal of Experimental Pathology, 13: 248-259, June, 1932.

2. Coburn, A. F., and Pauli, R. H, "Studies on relationship of streptococcus hemolyticus to rheumatic process; observations on ecology of hemolytic streptococcus in relation to epidemiology of rheumatic fever." Journal of Experimental Medicine, 56: 609-632, November, 1932.

3. Coburn, A. F., and Pauli, R. H., "Studies on relationship of streptococcus hemolyticus to rheumatic process; observations on immunological responses of rheumatic subjects to hemolytic streptococcus" Journal of Experimental Medicine, 56: 651-676, November, 1932.

4. Bunim, J. J., "Antistreptolysin titer in rheumatic fever, arthritis and other diseases." Journal of Clinical Investigation, 19: 75-82, January, 1940.

5. Randall, E., and Rantz, H. H., "Antistreptolysin 'O'; study of this antibody in health and in hemolytic streptococcus respiratory disease in man." American Journal of Medicine, 5: 3-23, July, 1948.

6. Hammon, W. M., Sather, G. E., and Hollinger, N., "Preliminary report of epidemiological studies on poliomyelitis and streptococcal infections; Lansing neutralizing antibody and antistreptolysin "O" surveys in California cities, Texas, North Carolina, Mexico, Pacific Islands, and Japan," American Journal of Public Health, 40: 293-306, March, 1950.

7. Breese, B. B., and Gray, H., "Antistreptolysin titer as aid in diagnosis of rheumatic fever." New York Journal of Medicine, 51: 389-391, February, 1951.

8. Rantz, L. A., Di Caprio, J. M., and Randall, E., "Antistreptolysin-O and Antihyaluronidase titers in health and in various diseases." American Journal of Medical Science, Volume 224, No. 2, 1952.

HELPFUL HINTS

Substitute—

The following technic may be substituted for marking numerous glass slides, which must remain in staining dishes, when a diamond-point pencil is not available:

The number of slides necessary for the smears are counted out and numbered. The patient's names are listed and given the same numbers as their slides. The NUMBERS are written with a wax pencil on the sticky side of a piece of adhesive tape. The tape is then placed and rubbed down tight on one end of each slide.

Since most adhesive tapes are water proof, the stain does not seep through to obliterate the numbers on the slides. This technic allows the slides to be transferred from one staining dish to another without confusion.

Reference: Marka Lee Duffield Smith, Grace Hospital Laboratory, Welch, W. Va.

Sedimentation Tube Holder—

A stopper from a 500 ml. Baxter Transfuso-Vac bottle, when inverted and set on the desk, may serve as a useful holder for one Wintrobe Sedimentation tube. The latter will fit snugly into the "Inlet" depression on the stopper and this unit may be set conveniently near your work where the tube can be constantly watched. Make certain, of course, that the tube is exactly upright; the tube fits very firmly and snugly into this particular stopper . . . When determinations of Sedimentation Rate are being done by a class, each member of the class can be given such a stopper to serve as a holder for this tube.

Reference: *Colorado Med Tech News*, Vol. 6, No. 3, Page 4, Feb., 1954

Colored Stoppers—

Cotton or sponge stoppers of needle holders can be colored with various stains in order to designate the size of the needles in the holders. In this laboratory, white stoppers are used for No. 21 needles—blue (Wright's Stain) for No. 22 needles—red (Safranin) for No. 23 needles—green (Malachite) for No. 25 needles—etc. Just a little stain on a swab transferred to the stopper makes it easy to select the proper size needle in just one second—and sometimes that second is a most important one.

Reference: Clementa Proudfoot, M. T. (ASCP), Myers Clinic Hosp. Lab., Philippi, W. Va.

A DESCRIPTION, DEMONSTRATION AND DISCUSSION OF TWO IMPORTANT SEROLOGIC TESTS*

NICHOLAS J. MENOLASINO, Ph.D.
Mount Sinai Medical Research Foundation, Chicago 8, Illinois

I. THE INFECTIOUS MONONUCLEOSIS TEST

Introduction

In 1911, Forssman reported that the injection of emulsion of guinea pig kidneys into rabbits gave rise to antibodies which reacted with sheep erythrocytes. This immunologic phenomenon depends on cross-reactive portions of antigens which are found both in animals and in many micro-organisms.

The blood sera of a large majority of people either naturally contain low titers of heterophile antibodies which agglutinate or hemolyse sheep erythrocytes, or abnormally, contain titers of these anti-sheep erythrocyte agglutinins of several types: (1) the Forssman antibody, found normally, which is of high titer after immunization of sensitive individuals with horse serum (this antibody titer is not elevated in infectious nononucleosis); (2) non-specific antisheep erythrocyte agglutinins found occasionally in the sera of individuals suffering from various diseases; (3) the heterophilic antibody of infectious mononucleosis.

The differentiation of the antibody of infectious mononucleosis from these other heterophilic antibodies is readily possible through the simple, selective absorption of the patient's serum with guinea pig kidney antigen and beef erythrocyte antigen. The results of this procedure are as follows:

(1) The Forssman heterphile antibody found in normal serum is completely absorbed with guinea pig antigen, but is not always absorbed with the beef erythrocyte antigen;

(2) The heterophile antibody found in the serum of persons injected with horse serum is usually completely absorbed by both the guinea pig kidney antigen, and the beef erythrocyte antigen;

(3) The heterophile antibody found in the serum of individuals with infectious mononucleosis is not removed at all, or only removed in part, by guinea pig kidney antigen, but is completely absorbed with the beef erythrocyte antigen.

Technic

I. Materials.

A. For the presumptive and differential tests:

1. Thirty 75 x 10 mm. test tubes for titration
2. Two per cent suspension in saline of sheep erythrocytes washed three times in physiologic solution of sodium

* Read before the Fall Meeting of the Illinois Medical Technologists Association, Chicago, Illinois, October, 1953.

chloride. The sheep erythrocytes should be at least 24 hours old, but not older than 2 weeks. They should be used only if the third washing gives a perfectly colorless supernatant. The cells should be washed and the suspension prepared and used in the same day.

3. Patient's serum inactivated for 30 minutes at 56°C
4. Positive control serum (correct interpretation will be facilitated by inclusion of a positive control serum from a known case of infectious mononucleosis).

B. For the differential test:

1. Two 85 x 13 mm. test tubes for absorption
2. Guinea pig kidney antigen.
3. Beef cell (erythrocyte) antigen.

Indications and Interpretations of Presumptive and Differential Tests

Test	Indication	Interpretation
I. Presumptive Test	1. Cases where clinical and/cr hematologic findings suggest infectious mononucleosis.	1. Diagnosis of infectious mononucleosis is highly probable if clinical and/or hematologic findings suggestive of infectious mononucleosis are present, and if the titer is 1:224 or higher. 2. If conditions mentioned under (1.) do not exist, or if patient recently received injections of horse serum, the presumptive test must be supplemented by the differential test.
II. Differential Test — A. Absorption with Guinea Pig Kidney Antigen	1. Titer of 1:112 or less as determined by the presumptive test, in cases suspected of having infectious mononucleosis. 2. Titer of 1:56 or higher in patients without clinical and/or hematologic findings of infectious mononucleosis. 3. A history of a recent injection of horse serum in a patient with a titer of anti-sheep agglutinins of 1:56 or higher as determined by the presumptive test.	1. Differential test for infectious mononucleosis is positive if the titer of anti-sheep agglutinins after absorption with guinea pig kidney antigen is not more than 3 tubes lower than the titer of the presumptive test.
II. Differential Test — B. Absorption with Beef Erythrocyte Antigen	1. It is advisable to do the absorption in every case with guinea pig kidney and with beef cell antigen. 2. Is essential in cases of patients with elevated titers of anti-sheep agglutinins if neither clinical nor hematologic findings are present, suggestive of infectious mononucleosis.	1. The test for infectious mononucleosis is positive in a serum in which the absorption with guinea pig kidney antigen failed to remove the anti-sheep agglutinins completely, and in which the beef cell antigen completely removed the anti-sheep agglutinins.

II. The Presumptive Test

Tube No.	1	2	3	4	5	6	7	8	9	10
Physiol gic saline, cc	0.4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Serum*, cc	0.1	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Serum Dilution	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
S.R.B.C.† 2% suspension, cc	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Final Dilution	1:7	1:14	1:28	1:56	1:112	1:224	1:448	1:896	1:1792	1:3584

* Mix and transfer 0.25 cc. to the second tube, etc., until the last tube is reached. Mix and discard 0.25 cc. from the last tube.

† S.R.B.C. = Sheep Red Blood Cells.

II. The Presumptive Test.

1. Incubation: at room temperature.
2. Time:
   (a) When speed is indicated, reading may be done after 15 minutes. If result is positive (agglutination at 1:224 or higher), the test may be considered completed except that the final titer will be higher after two hours' incubation.
   (b) If negative (titer less than 1:224), repeat reading at intervals as frequent as convenient
   (c) Final negative titer (titer less than 1:56) should not be recorded until after two hours' incubation. If speed is not a factor, it may be more convenient to read results at the end of two hours.
3. Reading the test: Results are read after shaking the test tubes to resuspend the sediment. Check with naked eye. If no clumping is visible, place the tube horizontally on the stage of the microscope and read with a low power objective (scanning lens—e.g., 25 mm., or 35 mm.) permitting viewing of the test tube.

III. The Differential Test

A. Absorption procedure with guinea pig kidney antigen:
1. Place in a test tube (85 x 13 mm.) 1.0 cc. of the thoroughly shaken suspension of guinea pig antigen.
2. To this, add 0.2 cc. of inactivated patient's serum.
3. Shake well and let stand for 3 minutes.
4. Centrifuge at 1500 r.p.m. for 10 minutes.
5. Remove the supernatant fluid carefully with a capillary pipette. Make sure to transfer only clear supernatant without particles. This supernatant is a 1:5 dilution of the patient's serum, absorbed with guinea pig kidney antigen.

B. Absorption procedure with beef cell antigen:
The procedure is the same as in A, except that the serum is absorbed with beef cell antigen.

IV. Titration of absorbed serum (after absorption with guinea pig kidney antigen or beef cell antigen).

The scheme outlined for the presumptive test is followed with one exception: to tube number 1, add 0.25 cc. of the supernatant fluid obtained from the absorption (absorbed patient's serum), and no physiologic saline. The reason for this is that 0.2 cc. of serum plus 1 cc. of the absorbing antigen results in a 1:5 dilution of the patient's serum, after absorption.

Incubation time, temperature and reading of the test are the same as for the presumptive test.

Possible Heterophile Anti-Bodies in Patient's Serum	I. Presumptive Test	II. Differential Test			
		A		B	
	S.R.B.C.[1] Agglutinated	Antibody Absorbed Out by G.P.K.A.[2]	S.R.B.C. Agglutinated After Absorption	Antibody Absorbed Out by B.C.A.[3]	S.R.B.C. Agglutinated After Absorption
(1) Forssman heterophile antibody in normal serum......	Yes	Yes	No	[4]	[4]
(2) Forssman heterophile antibody in serum sickness or horse serum sensitization........	Yes	Yes	No	Yes	No
(3) Heterophile antibody of infectious mononucleosis........	Yes	No[5]	Yes	Yes	No

[1] : Sheep Red Blood Cells.
[2] : Guinea pig kidney antigen.
[3] : Beef cell antigen.
[4] : Absorption is incomplete in approximately ⅓ of persons; if absorption is incomplete, agglutination is present.
[5] : Titer after absorption not more than 3 tubes less than titer in presumptive test.

II. THE RAPID SLIDE AGGLUTINATION TEST FOR SEROLOGIC IDENTIFICATION OF ISOLATED SALMONELLA AND SHIGELLA CULTURES.

A. The **Salmonellae**

Brief morphologic, biochemical and antigenic description:

The Salmonellae are gram negative, non-spore forming bacteria of a non-fastidious nature. Biochemically, they characteristically fail to ferment lactose and sucrose, do not liquefy gelatin and are indole negative. However, for purposes of closer identification, the biochemical characteristics are supplementary rather than confirmatory, and the accurate identification must depend on serologic results as well as biochemical reactions. Both types of findings must be carefully considered in the final identification.

The fact that the different species of Salmonella are closely related antigenically is applied in the Kauffmann-White immunologic scheme for classification of this genus. This scheme employs Roman numerals for the designation of the "O" or somatic antigens and lower case letters and arabic numerals for the designation of the "H" or flagellar antigens.

The antigens of this genus can be briefly characterized as follows:

I. Somatic antigens
  a. Thermostable
  b. Resistant to alcohol and acid treatment
  c. Show no "phasic" variation
  d. Show a finely granular type of agglutinate in the presence of specific antiserum

II. Vi antigen
  a. Occurs at extreme periphery of cell
  b. Usually encountered in freshly isolated strains

c. Can block the somatic agglutination
d. Destroyed by heating at 60°C for 1 hour
e. Destroyed by dilute acids and phenol
f. Lost by repeated subculture of organism

III. Flagellar antigens
a. Thermolabile
b. Destroyed by alcohol and acid
c. Show "phasic" variation and are therefore not as useful in rapid serologic identification of groups, but if identification of types is required it becomes necessary to type the "H" antigen as well.
d. Shows a loose floccular agglutinate in the presence of specific antiserum.

Major types of dissociation encountered in this group and their relationship to antigenicity:

1. Smooth (S) to Rough (R): the organisms become pan-agglutinable because the group specific somatic antigens are lost.
2. Vi to VW to W: loss of the Vi antigen
3. Motile to Non-motile: loss of motility associated with loss of flagellar antigen.

Description of the Rapid Slide Agglutination Test:

1. Obtain pure culture of suspected organism from:

   a) Primary stool plate (not recommended)
   b) Kliger's Iron Agar or Triple Sugar Iron
   c) Nutrient agar slant

   (b and c: secondary culture recommended)

2. Mark off into areas, about 1 inch square, a glass plate or bottom of a petri dish.
3. Add a drop of the particular antiserum (for use with rapid slide technic) to the square.
4. For each different antigen, to one square add a drop of physiologic saline. This serves as the saline control and its purpose is to detect non-specific or spontaneous agglutination, usually due to S R variation.
5. Next, add a drop of a heavy suspension of antigen to each square (suspension should appear "milky" for best results).
6. Mix the drop of serum and antigen together with bacteriological loop until homogenous and read for agglutination, which should occur within 30 to 60 seconds. The saline control plus antigen should remain smooth. If agglutination occurs in saline control, the antigen cannot be used.

Possible serologic scheme for **Salmonella** Rapid Slide Agglutination Test:

1. Polyvalent serum plus organism to be identified.
2. If serologic reaction with antigen plus polyvalent serum is positive, then the following group sera* are used in connection with the antigen to be identified: A, B, C_1, C_2, D and E.
3. If results are negative in each of the above titrations, and if biochemically the organisms resemble S. typhosa then:
   a) Vi serum antigen to be identified
   b) heat organism suspension to 60°C. for 1 hour and repeat the series of titrations in 2.
4. If the organism is in group "D", flagellar "d" antiserum can be used for closer identification

For a complete description and discussion of these various **Salmonella** groups, the reader can consult one of the excellent sources listed under selected readings in the bibliography.

B. **The Shigellae**

Brief morphologic, biochemical and antigenic description:

This group of organisms consists of gram negative, non-spore forming, non-motile small rods, which, like the Salmonellae, are non-fastidious. They ferment a variable number of carbohydrates with the production of acid, but no gas (exception, the **Newcastle** bacillus).

Although the antigenic pattern of the **Shigella** group is quite complex, grouping sera have been developed—but not for individual species identification. For this the organism should be sent to one of the enteric typing centers.

Since these organisms are not flagellated, the typing is limited to the somatic antigens, and the antisera are made up accordingly:

Group sera	**Has somatic agglutinin for:**	**Types**
A	**Sh. dysenteriae**	1-7
B	**Sh. flexneri**	1-6
C	**Sh. boydi**	1-7
D	**Sh. sonnei**	1-2
Alcalescens	**Sh. alcalescens**	1-2
Dispar	**Sh. dispar**	1-2

In using these grouping sera in the rapid slide agglutination test for **Shigella** identification down to the group, one precaution must be observed: there occurs at the extreme periphery of some organisms of the **Shigella** genus, an "envelope" antigen. If this antigen is present, the somatic agglutination, in the presence of the specific antiserum, will be blocked and consequently a false negative result will be observed. In order to circumvent this pos-

* These sera cover about 95% of all strains isolated in this area and are commercially available.

sible difficulty, cultures to be titrated against the **Shigella** group sera are routinely heated at 100°C for 30 minutes. This treatment eliminates the "envelope" antigen if it is present, and if it is not present, the somatic antigens of the cell are not altered in any way.

The technic for setting up the test is carried out in the same way as that described for the **Salmonella.**

Summary.

By the use of group typing sera for the **Salmonella** and **Shigella,** in conjunction with the biochemical findings, a simple, rapid and accurate laboratory diagnosis for the common enteric infections is possible. This method of serologic testing does not involve special or expensive equipment and consequently, is highly practical for the small diagnostic laboratory.

REFERENCES

1. Davidsohn, I., and Walker, P. H.: The Nature of the Heterophilic Antibodies in Infectious Mononucleosis. Am. J. Clin. Path., **5:** 445-465 (Nov.) 1935.
2. Davidsohn, I.: Serologic Diagnosis of Infectious Mononucleosis. J.A.M.A., **108:** 289-294, (Jan. 23) 1937.
3. Davidsohn, I.: Test for Infectious Mononucleosis. Am. J. Clin. Path., **8:** 56-60, (March) 1938.
4. Davidsohn, I., Stern, K., and Kashiwagi, C.: The Differential Test for Infectious Mononucleosis. Am. J. Clin. Path., **21:** 1101-1112, (Dec.) 1951.
5. Davidsohn, I.: Differential Diagnostic Problems in Infectious Mononucleosis. Wisconsin Medical Journal, 187-189, (March) 1953.
6. Dubos, Rene J.: *Bacterial and Mycotic Infections of Man*, 2nd Edition, Lippincott.

SELECTED READINGS

1. Edwards, T., and Ewing, W. H.: *A Manual of Enteric Bacteriology*, 1952.
2. Kauffmann, F.: *The Diagnosis of Salmonella Types*, 1950.

2a. ——————————: *Enterobacteriaceae*, 1951.

3. Leibowitz, S.: *Infectious Mononucleosis*, 1953.
4. Weil, A. J., and Saphra, J.: *Salmonellae and Shigellae*, 1953.

"THE ORGANIZATION AND OPERATION OF A BONE BANK"*

E. W. CULLIPHER, M.D., F.A.C.S.
401 Calumet Building, Miami 32, Florida

The organization of a Bone Bank is a practical and relatively simple procedure, which can be carried out in almost any hospital.

The idea of getting bone from a donor and transplanting it into a recipient patient, that is the homogenous bone graft, has been one of extreme interest to surgeons for many years. The idea of preserving this homogenous bone is not new. Ollier in 1867, Heitz-Boyer 1918, LaRich and Policard in France, Macewen 1912, in Great Britain, and Carroll all did work with bone banking and bone grafts. In the olden days of surgery, when the infection rate was very high, these attempts at transplantation of bone from one person to another almost routinely failed, and it became accepted as a fact that such procedure could not be carried out. In 1912, when two Frenchmen, Tuffier and Maggot, obtained good results from implanting bone that had been refrigerated for several months, the idea of homogenous bone grafting took on new life. It really became an accepted procedure after further isolated reports were made, in 1947, when Dr. Phillip Wilson and other American workers began to report their results.

Although Wilson has been most enthusiastic in some of his reports, there is almost no argument that autogenous bone, or the patient's own bone, is superior to anything else that can be used. It now appears that the homogenous graft is the next best thing, and in many instances, seems almost as good as the autogeneous graft. There are no specific indications when a Bone Bank graft is superior.

The advantages of a Bone Bank are that after sufficient stock is on hand, any type and amount of bone desired for a given operation is available. In children, many orthopedic operations, such as long spinal fusions and arthrodesis of a major joint, require more bone than the patient can actually spare from elsewhere in the body. The availability of a Bone Bank should then prevent failure of an operation because insufficient bone is at hand. In bridging bony defects, often times a very considerable amount of bone is needed, both the rigid cortical bone to maintain the length and position of a long bone, and cancellus bone to further fill in the defect and increase the speed of healing. On some of the so-called heavier orthopedic operations there is the ever present danger of surgical shock. If it is necessary to make another incision and further dissection to obtain bone to complete the primary operation, this further shock is impending.

* Read before ASMT Convention, June, 1954, Miami Beach, Florida.

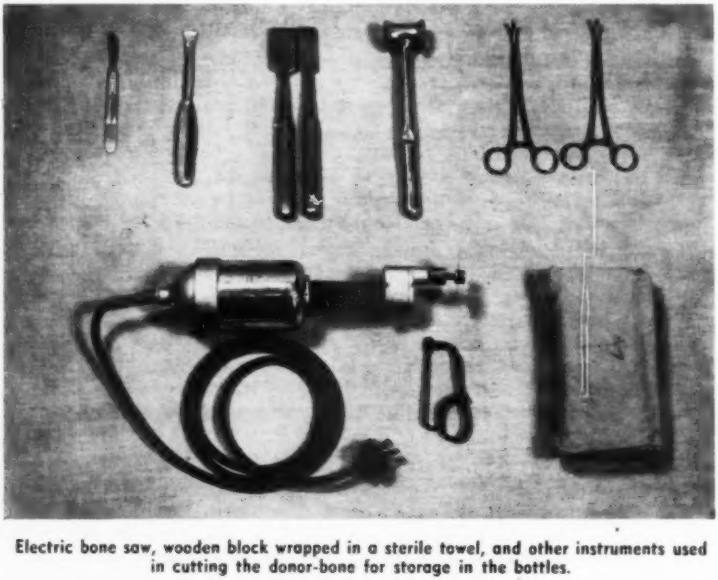
Not the least of our problems is that most patients, particularly women, do not want any more scars left than are absolutely necessary. As you can see from your visit here on the Beach, the hiding of an operative scar in a present day bathing suit is becoming quite a surgical feat to say the least. Some orthopedic operations have a rather high per cent of unsatisfactory results in the best of hands. This is particularly true of a lumbosacral fusion in the low back. It is now agreed that one factor which reduces this rate of failure is ample supply of bone at the time of the fusion, which can easily be supplied by the Bone Bank. Many Bone Bank specimens are then used in addition to what bone is available locally at the operative site, so that the supply may be ample or further re-enforcement done by the bone. When ample bone is available to the surgeon, it will undoubtedly be used more readily as a primary procedure with resultant increase in the satisfactory solution at the primary operation, rather than after many weeks of disability followed by a secondary operation and bone grafting. This is true particularly in certain types of fractures of long bones which require internal fixation, at which time added bone can be placed at the site of the fracture.

Various methods of preserving and storing bones have been used. The one most widely used today is the deep freeze method. The other next most frequently used is storing the bone in merthiolate. There has been some interesting experimental work done on vacuum-drying of bone by the Navy. This is a rather complicated process, more or less of an experimental nature, and may prove to be of specific benefit to the Services, but unless greatly simplified, can be used only in selected hospitals. It does offer the great advantage of being able to package the bone in such a way that it can be transported great distances with no difficulty whatever. In our hospital at Jackson Memorial, we use the deep freeze method.

The first problem in establishing a Bone Bank is, naturally, the source of the material. This comes from three main sources. The greatest source of bone in most hospitals is from amputations in living patients. The next most frequent source is bone obtained from living patients, in which it is removed for reason at operation, such as resection of a rib to enter the chest cavity, or resection of part of the wing of the ilium, in closing some incisions around the hip. The third source is bone from postmortems.

Much team work is necessary in the establishment and operation of a Bone Bank. We have notified all surgeons working at our hospital that if they are contemplating an amputation, we would like to be notified, so that we may inspect the limb and decide whether it is suitable for use as a source of bone to

be stored at the Bank. Where a limb is grossly infected, of course, none of the bone can be used because we do not attempt to sterilize any bone which is contaminated. After inspection of the limb, if it is decided that part of it will be suitable, permission is obtained from the patient to procure this bone for the Bank. Sterile bone preps are then ordered 24 hours in advance of the operation, if there is that much time available. At the time of amputation, the part is wrapped in sterile towels and under sterile technique the bone is removed from the limb in surgery. It is cleaned of all soft tissue down to the periosteum, which is normally saved where possible. It is then cut into lengths and sections, each of which should be stored in an individual bottle, in order that it may serve for one operation at a later date, rather than having a lot of bone in the bottle which would not be used but might be contaminated by excess handling. There is no objection to using more than one bottle at the time of operation, where large amounts of bone are needed. Each section of bone is then placed in a sterile bottle containing 30 cc. of Aureomycin. The bone is properly labeled as to the type of bone it is, that is what bone is was taken from, the patient's name, results of the Wasserman, or similar blood tests of the donor, and placed in the deep freeze where the



Electric bone saw, wooden block wrapped in a sterile towel, and other instruments used in cutting the donor-bone for storage in the bottles.

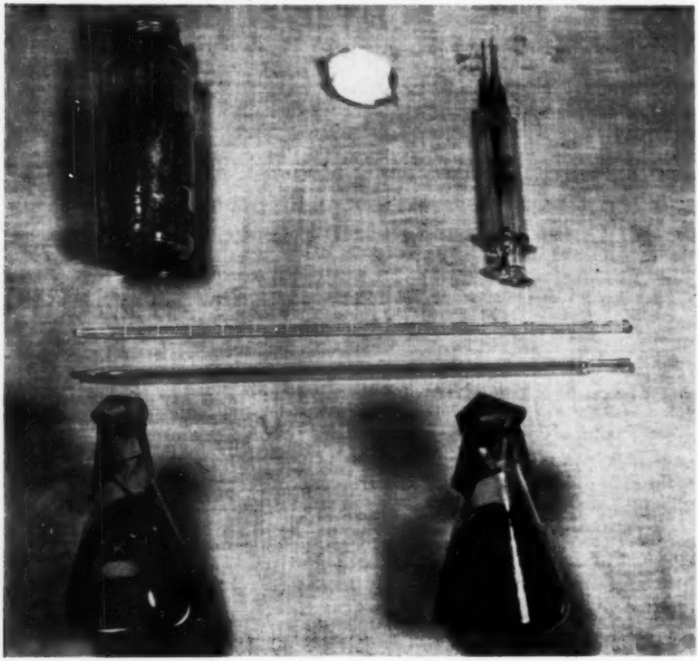
temperature is approximately minus 30 degrees C.

It is known that pathogenic bacteria thrive best in temperatures above 37 degrees C. and that their resistance to cold varies widely. Bacillus pyocyaneus can resist minus 75 degrees. Bacillus tuberculosis has been plunged into liquid air at minus 200 degrees, without any apparent damage. It has been shown that treponema can regain its motility and virulence after 4 months preservation in minus 78 degrees. Several strains of staphylococcus have been found active after storing at minus 30 degrees. Therefore, we do not feel that the cold is bacteriocidal, but that it is merely bacteriostatic. Viruses and spores appear to be practically unaffected by cold. This explains why we believe in rigid aseptic technique in taking and storing the graft, and that careful bacteriological control is essential. We do not expect this aureomycin to render the bone aseptic, although we hope it does have some tendency in that direction. It is used primarily as a solution which will come in contact with all the graft, and then when drawn out of culture, should give us a more accurate knowledge of whether contamination is present than taking a swab culture or even cutting off a small piece of the bone at one end.

It is believed that some importance should be attached to preserving the cellular integrity of the graft. This would undoubtedly be done better by rapid freeze method rather than just putting the bone in the deep freeze and allowing it to freeze gradually, which allows some separation of the water and salt, with the water freezing and thereby destroying the cells by formation of large ice crystals. Most investigators believe that any bone graft dies completely and is replaced by new bone by the so-called creeping substitution method. It is impossible under the microscope to state whether the bone immediately adjacent to the recipient bone has been replaced entirely by creeping substitution or whether a few of the bone cells live. However, it seems obvious that there are not sufficient cells surviving to make any appreciable difference. In any event, this is an entire study by itself, and we shall not go further into it at the moment. We have found that apparently the bone can be kept in the deep freeze almost indefinitely. So far I believe, we have not used any bone which has been stored over 2 years. This is primarily because we have never been able to keep 2 years ahead of the demand. We have been unable to tell the difference clinically from Bank bone which is relatively fresh and that which has been stored for several months, that is, up to 24 months.

After the bone has been stored in the Bank, the Bacteriology Department removes 1 cc. of this 30 cc. of aureomycin solution. This 1 cc. is put in Erlenmeyer flask containing heartbrain

broth. After 3 days, this is subcultured into another heartbrain broth mixture. 5 cc. are taken from this original culture bottle, which had 100 cc. By this dilution we have diluted our original aureomycin down to 1-2000. We believe that this great dilution will remove any inhibital factor on the culture. When the second bottle is reported sterile, which is usually about the end of 10 days, we consider the bone as safe to use and it is so labeled. There has been one Bone Bank that I know of which took no cultures whatever on any of its bone including some postmorten bone. Apparently, they had no more trouble than would normally be expected, but we feel that this is taking an undue chance with infection and resulting failure of the operation.



Bone stored in bottle with the solution and 2 flasks of culture media.

An ordinary commercial deep freeze is quite satisfactory for storing of the bone. While it is not necessary to use the entire deep freeze in a small bank, there are advantages of having no other material in the freezer except the bone and thereby hav-

Plasma bottle, rubber stopper and metal ring, rubber sheet, and large bottle of Aureomycin "bone solution."

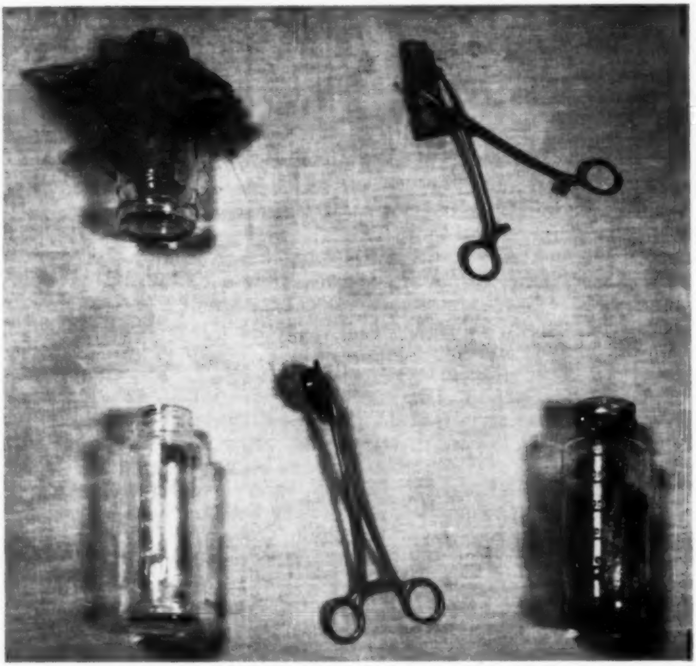
ing it disturbed as little as possible. We use ordinary plasma bottles with rubber stoppers, metal caps, with 6 inch squares of rubber gloves for storage. The plasma bottles, after being cleaned and washed, are sealed with rubber stoppers. The 6 inch square of rubber glove is tied over the stopper with an elastic band. The bottle is then autoclaved. The stopper, of course, must be put in very loosely, to allow escape of expanding air. The purpose of the rubber glove over the rubber stopper is to insure sterility of the top of the bottle, which is to be handled in surgery perhaps by the operating surgeon. This further insures the sterility of the neck of the bottle, both inside and out, and gives less chance of contamination when the bone is placed through the mouth of the bottle. Using a sterile syringe, an aureomycin solution is placed in the bottle before the bone. The bone solution used by us is 1/10 mg. aureomycin, 1/10 mg. of Chloromyecetin, and 100 units of penicillin per cc. in normal saline. The piece of rubber glove is removed, leaving sterile cap and neck for the surgeon to remove. The bone is

then placed in the bottle and the stopper is replaced. The metal cap is then screwed on the bone bottle. The bottle is labeled by the circulating nurse. On this label goes the name of the donor, age, surgical procedure, and a report of his serology; the type of the bone, that is whether it be tibia, fibula, etc., and the date of surgery is recorded. Blanks are left to be filled out by the Bacteriology Department and also to be filled out by the Orthopedic Surgeon at the time he uses the bone. In that way, we keep a continuous study on the results of the bone banking procedure.

If the Bone Bank is to be run efficiently, with the minimum of complications, a standard procedure is necessary, with specific instructions to each department that is cooperating in its operation. Our Central Supply has been given typewritten instructions on the method to pack the bottles. The bottles are then packed in Central Supply. The Surgical Nursing Staff has been given instructions on how to handle the bottles in Surgery. The Bacteriology Department has been given written directions on their part of the procedure. Written instructions on the method of handling all bottles has been sent to all the attending Orthopedic Surgeons in the hospital who use the bone. Periodically, we send letters to attending surgeons in the hospitals and inform them of our Bone Bank and our desire for bone. The Pharmacy has the formula for the solution and periodically makes it up a litre at a time, which is then stored in the Bank itself, and used as needed. We also have mimeographed forms which are sent to the Orthopedic Surgeons periodically to fill out and making followup studies on the individual grafting procedures. In that way, we hope that in time we will have sufficent statistical anaylsis to be of benefit to us and our patients.

Various containers have been tried for storing bone. A few years ago the most popular method was the so-called 2-bottle or 2-jar method, in which one jar is placed inside the other under sterile technique. This was thought to increase the sterility if there can be such a thing as degree of sterility, and lessened the chances of contamination. This is a somewhat bulky, bothersome method which has been rather largely discarded. Other places have used metal tubes.

We find the plasma bottle here described to have several disadvantages. The bottles are very seldom filled with bone. Therefore, a considerable amount of space is wasted. They are, of course, breakable, and if larger bottles are used, even more space is wasted, which soon becomes an item even though a large deep freeze may have been procured for the purposes of storage. The size of the bone must quite apparently be limited by the size of the neck of the bottle and forcing bone that fits



Rubber covered and stoppered plasma bottle, method of removing stopper and dropping bone specimen into bottle, and closed bottle ready for culture and storage in freezer.

too snugly through the mouth of the bottle is inadvisable because it may be overly difficult to remove and chances of contamination are greater.

For these reasons we are also seeking a better container. We have been experimenting with plastic bags 12 inches long and 6 inches wide. These bags are sealed in a sterile manner by a heating unit. One end contains a rubber stopper, through which the bone solution can be placed originally and withdrawn for culture. At the time of taking the bone, the end of the plastic bag is prepared with iodine or some other antiseptic, the bag is then cut across with sterile scissors, with the circulating nurse gripping the bag on each side, the mouth of the bag can be opened widely and even large pieces of bone can be dropped into the bag or removed. This opening is in marked contrast to that of a plasma bottle, and the amount of space that can be saved

by placing this plastic bag flat on the shelf is most advantageous. One bag can be stacked on the other because they are unbreakable and lie flat. In fact, the bone is taking up very little more space than its actual size, since if needed, or desired, the bag can be folded over the bone a time or two after it has been sealed in. We have encountered some technical difficulties with use of this method and have not as yet discarded our bottles.

We feel that our Bone Bank has been successful primarily because of the degree of cooperation of each one who has a part to play in its operation. There are many surgeons using the Bank, and while it is primarily a Jackson Memorial Hospital Bone Bank, bone has been used at other hospitals in our community. So far, we have made only one attempt to remove post-operative bone. At this time, we obtained a large amount of very desirable bone, and our Bank account took a very nice rise. However, our enthusiasm soon had a rude awakening when we found all this bone to be contaminated with B-coli, although the bone was removed within 2 hours after death of the patient. The entire amount was therefore discarded. Some Banks have reported sterilizing contaminated bone by various methods after which, of course, they have a very careful bacteriological control to prove that their sterilization has been adequate. We have not tried this up to the present time. The most available sterilization would be either boiling or autoclaving, which was done many years ago for the first time and after considerable experiment in various laboratories, it was very definitely shown that this bone was not desirable as a grafting material, although there were some successes in using it. Resected ribs are available in varying amounts in most hospitals where the resection is done in a clean case with no infection, particularly no tuberculosis and no malignancy. However, for some unexplained reason, rib bone is rather poor, and has a high rate of absorption when used as a graft. At least grossly, it appears to be ideal bone because of the thin cortex and a relatively large amount of cancellus bone with it.

Accumulating our followup studies on the final result of bone graft bone, the individual surgeon usually carries out the majority of his final after care in his office and we must, therefore, depend upon him to send us records. In our recent attempt to analyze the results up to the present time, we have had rather poor success in having these completed records returned to us. A higher per cent of our patients are transients or visitors, who leave our community before a final result can be obtained. We have had up to the present time, only 33 cases which we feel have complete and adequate followup. It is realized that bone grafting operations require many months to reach maximum

improvement and final rehabilitation and since our Bank is only a little over 3 years old, we could not have too many of our final results available.

There are several factors which may well be considered when assessing the final result of Bone Bank bone. In the first place, any operation requiring bone graft following delayed union or non-union indicates that the circulation at the site of this failure of union is not good. Bone Bank bone is likely to be used where the defect is large and would, therefore, naturally be harder to heal. Non-unions have a relatively high per cent of failure of bone graft whatever the material may be that is used. Oftentimes this Bank Bone has been used in addition to the other bone, particularly in fusion of the lumbosacral spine, which usually has a failure rate of approximately 20%. Failure means either absorption of the graft, fracture of the graft, or infection and sometimes complete sequestration of the graft. We have, as a final figure, 72% success. This compares favorably with the results of most Banks as the figures have been published. Of this percentage of failures 6.6% were due to infection, most of which had been present prior to the surgery and had been re-alighted by surgery. There were 12% which had not actually reached maximum improvement although the result was delayed. Adding these two figures then, the complete failure of the graft bone as on a basis of circulation or, revasculation of the graft, was 9.4%.

Our greatest failure rate was in the ununited fractures, as is true of any type of bone graft. Our statistics so far, reveal that we have had only 69.9% completely satisfactory. The other failures were due to infection or absorption as stated above. In some conditions, such as filling bone defects, while we have had very few, there have been no failures. In arthrodesis of the spine and major joints, there have been two failures. Most of these have been rather long spinal fusions for scoliosis in children. Where osteotomy has resulted in some defect in bone, which has been filled in by Bone Bank bone, we also have no failures at the present time.

I have attempted to bring to you an outline of the Bone Bank, as organized and operated at Jackson Memorial Hospital. We claim no originality for this Bank and have quite openly and frankly copied better features of other Banks previously established in various parts of the country. The alterations or variations, as applied by us, are relatively minor. Some points as outlined here, could well be open for discussion. However, we have found that this Bank operates simply and efficiently for us and we are, therefore, satisfied. The success of the Bank is a community effort of the hospital and without each person who is involved in this operation doing his job meticulously, we

would certainly be unsuccessful. As in all of medicine, the Medical Technologists, which in this case is in the Bacteriology Department plays a vital role and we must depend upon them completely. Any hospital that does bone operations can use a Bone Bank to advantage, even in some operations which had been begun without planning the use of further bone.

BIBLIOGRAPHY

1. Abbott, L. C., Saunders, J. B., and Best, F. C. (1947) : The evaluation of cortical and cancellous bone as grafting material. Journal of Bone and Joint Surgery, 29, 381.
2. Bosworth, David M., Wright, Howard A., Fielding, J. William, and Goodrich, L. Raymond (1953): A study in the use of bank bone for spine fusion in tuberculosis. Journal of Bone and Joint Surgery, 35-A, No. 4, 329.
3. Campbell, W. C.: Operative Orthopedics, C. V. Mosby Co., St. Louis.
4. Campbell, Crawford J., Brewer, Thomas, Macfadden, D. Glen, Payne, Edward B., and Doherty, James (1953): Experimental study of the fate of bone grafts. Journal of Bone and Joint Survey 35-A, No. 4, 332.
5. Herbert, J. J. (1951) : Homografts and bone bank. Journal of Bone and Joint Surgery, 33-B, 316.
6. Kreuz, F. P., Hyatt, G. W., Turner, Thomas, and Bassett, Andrew L. (1951) : The preservation and chemical use of freeze-dried bone. Journal of Bone and Joint Surgery, 33-A, No. 4, 863.
7. Meiss, W. C. (1951) : Use of calf bone bank. Journal of Bone and Joint Surgery, 33-B, 466.
8. Reynolds, F. C., and Oliver, D. R. (1949) : A clinical evaluation of merthiolate bone bank. Journal of Bone and Joint Surgery, 31-A, 792.
9. Weaver, J. B. (1949) : Experiences in the use of homogeneous bone. Journal of Bone and Joint Surgery, 31-A, No. 4, 778.
10. Wilson, P. D. (1947) : Experiences with a bone bank. Annals of Surgery, 126, 932.
11. Wilson, P. D. (1951) : Follow-up studies of the use of refrigerated homogeneous bone grafts in orthopedic operations. Journal of Bone and Joint Surgery, 33-A, 301, 307.

COMMON ERRORS IN LABORATORY TECHNIQUE*

GRETCHEN SQUIRES, M.D., *Pensacola, Florida*

There are three main sources of error in the performance of diagnostic laboratory tests:

1. Error due to fundamental lack of knowledge of instruments used.
2. Technical error due to methodolgy.
3. Human Error.

I. Errors Due to Fundamental Lack of Knowledge of Instruments Used.

I am constantly surprised at the inability of the average technologist to do simple adjustments on the instrument that they use most commonly—the microscope. Dirty microscopes are the rule rather than the exception to the rule in the average laboratory. This is possibly due to the fact that a number of individuals are using the same instrument and no one feels a personal responsibility for its upkeep. Yet, a dirty microscope is a poor tool for the exacting work required of it. How many of you have read the booklet that came with the microscope *you* use to discover its improvements, its new gadgets that will help you with your work, and its normal maintenance? Those of us who have purchased our own microscopes and who have been personally responsible for their care, in many instances are using microscopes purchased 15 and 20 years ago. Yet the average life of a laboratory microscope is 5 years. This is not due to any difference in the initial quality of the apparatus, but only in its aftercare. May I please remind you that you are also responsible for the care of the microscope you use because upon it *you* are dependent.

Next to the microscope, the instrument most commonly abused, misused and misinterpreted is the laboratory photometer. It may be wise, at this point, to stop for a moment and review briefly what is meant by the terms photometer and colorimeter as most of us, myself included, misuse the term colorimeter. To avoid confusion, it appears desirable to define a colorimetric procedure as one in which the colored solution representing the substance in unknown concentration is brought to exact color match with the standard color representing the substance in known concentration, while a photometric procedure is one based upon the direct measurement of color intensity in terms of the light-absorbing power of the solution at a specific region of the spectrum. Unlike colorimetric procedures, which are limited to the visible portion of the spectrum, the general principles of photometric procedures are as applicable

* Read before ASMT Convention, June, 1954, Miami Beach, Florida.

to the absorption of energy in the ultra-violet and infra-red portions of the spectrum as they are to absorption in the visible region. An increasing analytical use is being made of this fact. A good technologist routinely checks himself and his instruments by the running of standard solutions. The peace of mind acquired by running routine standard solutions at intervals and checking the calibration of your photometer is well worth the slight cost of the standard solutions used. The American College of Pathologists has for sale, through a Standards Bureau, sealed ampules of the common chemistry solutions for these standardization procedures if such solutions are not available locally. When we consider that the photometer is an electrical device that is prone to develop personal quirks from time to time we must recognize the necessity for recalibration at intervals. Any competent technologist should know how to set up a calibration curve for a new technique that is not included in the manual.

The choice of photometers from the many types commercially available is largely a question of the requirements of the individual laboratory. For routine analytical purposes based upon established procedures any good type of filter photometer will prove satisfactory. If a single phototype cell instrument is desired, the stability on the laboratory current should be tested before use or facilities for maintaining a storage battery must be available. For analytical purposes a spectro-photometer covering only the visible and neighboring portions of the spectrum is little better than a good filter photometer, except for the convenience of wave length selection. For investigational purposes, the spectro-photometer should be usable in the ultra-violet and infra-red regions as well as the visible region. An ideal combination would include such a spectro-photometer for investigational purposes and a good filter photometer for analytical purposes. Most good physiological chemistry books give, with each method, a definite known standard. It is wise to run such a standard along with the test determination, either routinely in setting up a new procedure, or at intervals using a standard procedure.

Which brings us to a point that may seem extremely fundamental and to many of you even a little insulting and that is the preparation of solutions. The faulty preparation of solutions is a constant and unfortunately, all too commonplace cause of laboratory error. To my mind, a course in quantitative analysis is one of the most important basic requirements recommended by the Registry of Medical Technologists (ASCP). For the determination is no better than the solutions used in its accomplishment and without an ability to weigh materials properly, to measure materials properly and to calculate their proportions, the final solutions used will be in error and the final results of

the procedure will be similarily erroneous. I think that the reason so many technologists either dislike or are apprehensive regarding the chemistry desk in a general laboratory is that they personally feel a fundamental lack in their ability, not to do the procedure itself, but to calculate its results. To many of us, myself included, the higher aspects of chemical formula calculations are unknown territory and best to be avoided, but the good basic chemistry texts that have been written for laboratory determinations usually give an adequate explanation of the necessary calculations and the sources of error of each procedure.

It sounds rather trite to mention laboratory glassware as a common source of error; yet no determination is any better than the glassware with which the determination is done. If the preparation room is turning out, for general use, glassware that has been improperly cleaned, pipettes with tips broken off so that measurements are less accurate, pipettes that are still wet, etc., the work of the laboratory will suffer accordingly. Many of us feel that the preparation room is practically in the housekeeping department and have gone to very little trouble to instill in its personnel a feeling that they, too, share the laboratory's responsibility to the patient. Again, good technical procedure boils down to an awareness by all concerned in the performance of the test of their personal responsibility toward the medical care of the patient.

II. Technical Error Due to Methodology.

The selection of a method of procedure is at times a difficult task because so many are available and the inexperienced individual is at a loss as to which of a group is most suitable for their use. In many instances, where we lack the proper knowledge for the selection, it is best to rely upon those methods specifically given in standard texts of laboratory procedure. This may occasionally lead us astray, because one individual may constantly reprint a method that has become obsolete, but at least it will be one that has stood the test of time and will suffice until we can educate ourselves to the choice of a newer method. Such existing methods should not be lightly discarded unless the new method promises at least a 25% improvement over the old in simplification of technique or in improvement of results. The loss of years of cumulative experience incurred by the change is such a serious drawback that it may completely offset the potential advantages of the new method. Such changes also discourage the collection or vitiate the value of long-term statistics. It is unwise, therefore, to introduce a new method without lengthy "overlapping" with the old. This will prevent premature unwarranted change and stabilize the laboratory organization.

(A) *Hematology.* The majority of patients who enter a hospital undergo one and often many, hematological investigations. If some of these tests are carried out repeatedly, even on the same normal patient and by the same technologist, appreciable differences occur in the final results. These differences are due to technical and sampling errors and may be greater than the changes by which the clinician wishes to distinguish normal from abnormal. It is important to know both the extent and variability of this technical error in commonly used tests.

1. *Hemoglobin Determinations.* In clinical hemoglobinometry the marked demand for simplicity has resulted in the use of a number of archaic instruments and in methods showing a considerable degree of error. The Medical Research Council of the British Empire in 1941 set up a sub-committee to deal with hemoglobinometry and a comprehensive investigation of methods and their results were carried on for seven years. As a result, there is now available a central reference standard of known hemoglobin values and means for the accurate standardization of any instrument or method. Those responsible for this work would be the last to claim that it has solved the problem, but it may be hoped that a definite raising of the standard of clinical hemoglobinometry will follow. It is generally conceded that a hemoglobin determination done by photo-electric hemoglobinometry will yield a more uniform figure when done by varying peoples at various times than will any other single method. The main advantage is the elimination of the human element and the accuracy with which the results can be obtained apparently with the minimum of effort. In practice the use of such equipment requires considerable care and these photometers must be serviced and standardized at regular intervals. Now that the Medical Research Council Committee has provided a carboxyhemoglobin standard which though practically permanent, was defined in trichromatic terms so that the color could, if necessary, be reproduced at any time, it is practical to have adequately calibrated instruments. What is needed now, and there may be a source of such material of which I am ignorant, is a commercially available preparation to allow us to recheck our instruments at intervals. Another point of definite interest is the confusion that exists about the previously established normal hemoglobin values. As many of you know, the Sahli standard is the lowest according to the present carboxyhemoglobin standard, being a rough equivalent of 86% of normal and the Haldane scale has an equivalent of 107% with the other authorities being between these two values. This allows us to rationalize more easily the marked differences in the average normal as recorded from, for example, England and the United States. In England the use of the Haldane standard was commonplace—in the United

States Sahli was preferred so that our overall picture appeared to give us a population group with a higher percentage of hemoglobin than in the British Isles. Considering that the estimation of hemoglobin is made with an extremely small sample of the 5 liters of blood contained in the whole body, it has been shown that considerable variation of hemoglobin readings occurs in different capillary samples taken at the same time and at varying times during the day. Taking all these factors into account it is unlikely that the method would have a co-efficient variation of less than 5%. This total error is composed of a technical error, including that due to pipetting and reading, of about 1.6% and a sampling error of about 4-5%. It should be emphasized that the component, due to reading of the photometer is about 1%. The use of the photometric colorimeter which may have a slightly smaller error when used by a skilled technician, cannot reduce the total error of the routine method.

2. *Hematocrit.* There is a growing tendency, on the part of clinicians, to be more dependent upon the packed volume of red blood cells i.e. the hematocrit, than upon the hemoglobin and red blood cell determinations. It is definitely true that the percentage of error in the performance of a hematocrit is much less than is inherent in the methodology for hemoglobin and red blood cell determinations, and from a practical point of view, the hematocrit is, in most instances, of equal clinical value. Using a single sample of blood, the packed cell volume can be measured with a co-efficient variation of 1%. From the technical point of view, this is the most accurate of hematological investigations. In practice, the error is larger than 1% because of the variation which arises from the sampling and pipetting error. Since the sampling error is of the order of 4-5%, the co-efficient variation of hematocrit is of 4-5%. This error is probably proportionable to the reading.

3. *Red Blood Cell Count.* It has been shown by numerous authors that the sources of error in the red cell count are large and numerous. As with the hemoglobin information, variability is introduced by the calibration of pipettes and dilution of the blood sample. These errors are larger in red cell counting, because a smaller volume of blood is used. An additional source of error is introduced by the use of different counting chambers and, as in any procedure involving counting, by the uneven distribution of the cells counted. Finally the sampling error due to the use of small volumes of blood must be important and different observers tend to count slightly differently. It has been calculated that in the routine use of the test it is very unlikely that the total co-efficient of variation would be less than 10% for a count of 5,000,000 cells per cubic mm. with 1-200 dilution of blood and counting over 80/400 square mm. of the counting chamber. It is noteworthy that the error due to the random

distribution of the cells varies according to the number of cells counted, being greater as the numbers of cells counted decreases. I would like to refer you, at this point, to an extremely interesting article in "The American Journal of Clinical Pathology," Vol. 21, p. 973-978 of October, 1951, in which the author reviews three methods of counting RBC with the calculated effect of the so-called "rejection" procedures on the total accuracy of the blood counts. As many of you were taught, myself included, where a square did not contain a relatively equal number of red blood cells, the entire square was rejected and another was counted in its place. It is interesting to note that the total counts were much less accurate where such squares were rejected than if squares were counted at random. The deviation anticipated was the square root of M ($\sqrt{M}$) if M is the mean count per square (i.e. 81 gives a deviation of plus or minus 9%).

4. *Hematological Indices.* As you may gather from the previous information, the hematological indices are subject to the same range of error as previously described since they are based upon calculations including the hemoglobin and red blood cell values. The main point that I am trying to stress is that the hematologic calculations which we present to the clinician are relative and not absolute figures and should be used as such. Only by research methodology with constant repetition of counts on the same sample and on various samples, with calculations by formulae derived from co-efficients of variations can a relatively absolute value be given and this is obviously impractical in routine laboratory procedure.

5. *Differential Counts.* To understand the inherent error present in the routine counting of 100 white blood cells, on even a well made and stained blood smear we need only to review some of the statistics acquired by studies at the University of Tennessee School of Medicine. White blood cells from routine laboratory slides were photographed and these blown up photographs were numbered and studied by the personnel of the hospital and medical school laboratory and faculty. An average error of ±5% was noted, with the largest degree of error occurring in the differentiation between the juvenile, stab and segmented neutrophile. In reviewing the usual teaching texts, there is considerable confusion as to, not only the terminology to be used for these cells, but their actual physical characteristics. The only solution I personally have been able to reach in this matter has been to choose one terminology and to familiarize the laboratory personnel and the attending physicians with the terminology being used. At the present time, we are using the nomenclature as given by Dr. Custer in his "Atlas of Blood and Bone Marrow" in which a *juvenile cell* is described as any cell of the granulocytic series having specific granules in the cyto-

plasm and a nucleus intermediate in shape between the rounded or oval non-indented nucleus of the myelocyte and the curved or coiled band of the stab cell. The nucleus of the juvenile cell has an indented oval shape resembling a bean or kidney. A *stab cell* is specifically defined as any cell of the granulocytic series which has a nucleus that can be described as a curved or coiled band no matter how marked the indentation, if it does not completely segment the nucleus into lobes connected by a filament. It is differentiated from the juvenile cell by an appreciable length of the nucleus having parallel sides and from the segmented neutrophile by having no indentation which could be described as a filament. It is possible that in order to simplify terminology an eventual classification will be arrived at in which the juvenile and stab cells are grouped together to include all cells of the granulocytic series between the truly segmented neutrophile and the rounded nucleus of the myelocyte. This is commonly used in bone marrow studies where the juvenile and stab cells are grouped together as metamyelocytes. Since the presence of stab and juvenile cells merely indicates a shift toward immaturity, this would seem to be a logical step. The point that should be emphasized for the differential white blood cell count, however, is the counting of an adequate number of cells to arrive at an accurate differential. In routine laboratory work the counting of 100 cells is usually sufficient. If, however, the differential count is abnormal in any cellular aspect, an additional 100 cells should be counted from another area on the same or on a different side, with the final count being expressed in terms of 100 cells.

6. *Fragility Tests.* The methods for demonstrating increased fragility of erythrocytes are extremely simple if one fundamental fact is remembered, that is, the accuracy with which the salt solutions are measured. These results are being reported in figures of 0.02% and a very slight error in the original solution gives a completely false result. Despite the known stability of sodium chloride solution, evaporation is common, particularly in warm climates or in cabinets exposed to radiator heat, and fresh salt solutions should be made frequently to prevent any false figures being reported. Remember that the diagnosis of hemolytic anemia is often based on the results of this one test so that the patient's treatment is predicated upon the accuracy of your work.

7. *Urinalysis.* Despite its long usage without fundamental change, and in spite of the accuracy with which the component tests of a urinalysis can be performed, there is no laboratory field in which less total accuracy is shown. A review of the examinations given by the Registry of Medical Technologists (ASCP) reveals more failures and a greater percentage of error in the urinalysis section than in any other segment. Since the laboratory proce-

dures are relatively simple and well worked out, this degree of inaccuracy can only be attributed to human error. It is probable that the reason for these constant inaccuracies lies in the attitude of the technologist toward the procedure. I personally feel that this attitude has been unconsciously fostered by each succeeding generation of technologists. As a student, each technologist in turn has been allowed to take over the urinalysis desk as a first assignment, because of the relative simplicity of the determination. It has always been felt that anyone who can see and write is capable of doing the preliminary steps of a urinalysis—that is, the specific gravity, color, reaction and character of the urine. Since the advent of the testing tablets for albumin, acetone, bile and sugar, these, too, have been relegated to the untrained help. Little attention has been paid to instructing this untrained help as to the importance of their determinations and the accuracy with which they must be performed. Only the microscopic urinalysis is left for the trained technologist and this, too, soon passes into the hands of the student after a very brief period of instruction. Yet, from the clinician's point of view, the results of an accurate urinalysis may be the key to a correct diagnosis in a puzzling case, and the recording erroneously of a negative urinalysis may change the entire treatment of a given case. The fact that the Registry Examinations reveal so many inaccuracies and mistaken ideas among the theoretically trained student indicates that we as teachers have fallen down badly in this particular section of laboratory procedure. It is difficult, I know, to develop at this late date a greater sense of responsibility toward the performance of the routine urinalysis, but the fact remains that it must be of great importance or it would not be one of the three tests required by the American College of Surgeons on each patient admitted to an approved hospital. I think the time has come for us to re-evaluate our attitude toward the urinalysis.

8. *Stool Examinations.* The examination of the routine fecal specimen is the most disliked job in the laboratory. This is, I feel, primarily due to its odor rather than to an inherent objection to performing the test, but the fact remains that a laboratory will use any possible strategem not to accept a stool specimen and once one has been accepted will relegate it to a side table to be done only as a last resort. Yet in our own part of the world we know that when stool specimens are properly examined, approximately 20% of the general population show intestinal parasites of pathologic significance. If a laboratory is going to do stool examinations there must be adequate attention paid to these specimens and, above all, adequate training of the technologists concerned. Many of our otherwise competent technologists are extremely deficient in parasitology training al-

though parasites are constant inhabitants of the intestinal tract of our everyday population. This brings to mind a story that would be funny if it were not so tragic. In a large eastern city approximately 15 years ago I had occasion to check the hospital's laboratory regarding their procedure for doing stool examinations. On two separate rectal biopsies I had found amoeba in the biopsy specimens and yet the stools were being consistently reported as negative. I found the personnel literally screening the stool by forcing it through a piece of screen wire. If no parasites were left on top the stool was reported as negative. Once the personnel was adequately trained a surprisingly large number of parasitic infestations were discovered. All stools should have a direct smear, a concentrated smear and a zinc sulfate flotation examination before being reported as negative. May I emphasize the necessity for constant checking of the specific gravity of the zinc sulfate as minor changes in concentration affect the accuracy of the determination.

III. Human Error.

This in turn may be subdivided into errors of commission and errors of omission. The errors of commission include mechanical errors in technique that are usually the result of carelessness, haste, or lack of fundamental knowledge of the procedure. The errors of omission are much more dangerous because they reflect a lack of technical integrity. The individual who through lack of personal honor will report an examination that has either not be done, or has been improperly done, with knowledge of the fact, is not worthy of the name of either technician or technologist. These are the fundamentally dishonest individuals who unfortunately crop up in any profession, technology not excepted. The best safeguard against this type of practice occurring in any laboratory is a thorough understanding by the laboratory personnel at all levels of the fundamental importance of the work they are doing. I fail to see how an individual knowing that on the basis of their white blood count and differential examination a decision will be made to operate or not to operate on a patient, could, because of the hour of the night or the personal felings of the technician, do a differential of only 25 cells and multiply by four for a total count or to count one large square on the white blood chamber instead of four, or in pipetting the blood into the pipette originally, go above the mark without starting over. A good technologist is as dedicated to humanity as is a good doctor or a good nurse. You are professional people and by acceptance of the pre-requisites of being a professional person certain responsibilities must also be accepted, one of which is personal inconvenience. The mere granting of a degree in medical technology or acceptance by the

Registry of Medical Technologists does not guarantee technical integrity. But fortunately the caliber of individuals who are willing to make the personal sacrifice, who are willing to study long and hard for the attainment of this degree weeds out a large proportion of those who are seeking the field only for the prestige it offers. Like the nurses, technologists are an underpaid profession and in many areas are not even recognized as yet as a profession. It is up to the American Society of Medical Technologists which you represent and to your counselor pathologists to make the MT (ASCP) emblem one of which all of us can be proud.

SAFETY MEASURES

Keep all flammable liquids in safety cans bearing the stamp of approval of the Underwriters Laboratories—and approved by the local fire department. These are obtainable from large hardware dealers. Keep nothing larger than one-gallon approved safety cans of flammable or volatile liquids in the laboratory. Larger stocks should be kept in the approved storage rooms, properly ventilated to take care of fumes and heat, as well as provided with fire doors.

Small or one-quart approved safety cans should be used on the shelves for routine dispensing of flammable or volatile liquids. Pouring of such liquids should be performed inside the fume hood with the blower in operation.

Blood diluting pipettes should be washed and dried (if volatile liquid such as acetone is used) inside the fume hood, with the blower in operation.

Matches should be kept in metal containers with lids.

Shelves should be checked periodically for smaller bottles of highly volatile reagents to be certain no one has failed to return them to the refrigerator.

Before lighting the Bunsen burners, check to see that no volatile liquids are being used at the same time and turn them off when not in actual use.

Keep stock paraffin in metal containers instead of the usual cardboard containers in which they are purchased.

(These rules were gleaned from an article written by a Medical Technologist **after** a fire in her laboratory.)

From the *Techni-Caller* (Arkansas) Vol. 3, No. 2, 1954 issue.

A RAPID ESTIMATE FOR DETERMINING SODIUMS AND POTASSIUMS BY FLAME PHOTOMETRY*

JEANETTE SANDUZZI, B.A., M.T. (ASCP)
Mountainside Hospital, Montclair, New Jersey

With the advent of the Flame Photometer sodiums and potassiums may be determined within a shorter space of time than formerly by chemical methods. However the Flame Photometer is still not run as a "Stat" procedure on Sundays, Holidays, Nights, etc., when only one technician is on call or duty; because the procedure still takes about 20-30 minutes of time for one technician to perform. Nevertheless there are times when the sodium and potassium value is of the utmost importance to the physician and would therefore be required as a "Stat" procedure.

To overcome this difficulty I have determined a method whereby both sodium and potassium values may be estimated quite accurately within less than five minutes time on the Janke Flame Photometer.

Because of the 10% variability of the sodium and potassium curves from day to day it has been necessary to construct a new sodium and potassium curve from a number of standards of both sodium and potassium. This of course is time consuming but necessary.

However I have found that a master curve, one for sodium and one for potassium may be constructed from the various standards and be used for estimating both values until a new batch of lithium need be made up. Therefore the one curve can be used for a period of months depending on how long your lithium standard lasts. A calibration chart may even be prepared from both these curves, one for sodium and one for potassium, to simplify calculation.

Procedure

After the customary warming up period of the Flame Photometer, the sodium and potassium value may be obtained very rapidly.

You will have already constructed your master curves for both sodium and potassium and they are therefore ready for use.

For sodium determination set your filter on Na, open your funnel dial and turn on your air pressure. Select a normal sodium standard, such as the 0.70 mEq./liter. Set your balance dial on the reading the master curve shows for this value. Then pour the selected standard into your funnel and as it empties, turn your zero adjustment dial until your galvanometer registers zero. Keep adding this standard until the zero reading is constant. Leave the zero adjustment dial on the number you have

* Read before ASMT Convention June, 1954, Miami Beach, Florida.

Comparisons of Sodium and Potassium by Two Techniques

		SODIUM TABLE			POTASSIUM TABLE		
No.	Date	Na.—mEq./L. Daily Curve	Na.—mEq./L. Master Curve	Difference	K—mEq./L. Daily Curve	K—mEq./L. Master Curve	Difference
1	9/24/53	140	140	0	3.75	3.50	—0.25
2	9/25/53	144	144	0	3.5	3.75	+0.25
3	9/25/53	150	152	+2	3.5	3.5	0
4	9/26/53	138	138	0	4.5	4.75	+0.25
5	9/26/53	140	140	0	3.0	3.0	0
6	9/28/53	140	144	+4	3.75	4.0	+0.25
7	9/28/53	138	140	+2	4.0	4.0	0
8	9/29/53	142	144	+2	3.5	3.5	0
9	9/30/53	148	148	0	3.5	3.5	0
10	9/30/53	140	140	0	4.75	4.75	0
11	9/30/53	132	130	—2	4.0	4.0	0
12	10/ 1/53	140	140	0	2.75	2.5	—0.25
13	10/ 1/53	132	134	+2	2.5	2.75	+0.25
14	10/ 1/53	136	138	+2	3.0	2.75	—0.25
15	10/ 2/53	134	136	+2	....	....	......
16	10/ 2/53	148	148	0	3.5	3.75	+0.25
17	10/ 2/53	148	148	0	3.5	3.25	—0.25
18	10/ 2/53	142	140	—2	4.0	4.25	+0.25
19	10/ 2/53	148	150	+2	4.5	4.75	+0.25
20	10/ 5/53	154	154	0	2.5	2.75	+0.25
21	10/ 5/53	134	134	0	....	....	......
22	10/ 5/53	140	140	0	....	....	......
23	10/ 5/53	152	152	0	....	....	......
24	10/ 5/53	154	154	0	3.75	3.5	—0.25
25	10/ 6/53	140	140	0	3.25	3.25	0
26	10/ 6/53	132	132	0	4.0	4.0	0
27	10/ 6/53	152	152	0	3.0	3.0	0
28	10/ 6/53	140	140	0	3.0	3.25	+0.25
29	10/ 7/53	132	132	0	....	....	......
30	10/ 7/53	140	140	0	....	....	......
31	10/ 7/53	118	118	0	5.25	5.25	0
32	10/ 8/53	140	140	0	....	....	......
33	10/ 9/53	114	112	—2	....	....	......
34	10/ 9/53	144	144	0	....	....	......
35	10/ 9/53	142	142	0	3.5	3.75	+0.25
36	10/10/53	152	152	0	3.25	3.25	0
37	10/10/53	...	...	..	5.0	5.0	0
38	10/13/53	144	144	0	3.5	3.5	0
39	10/13/53	136	136	0	3.0	3.25	+0.25
40	10/13/53	144	144	0	3.75	3.75	0
41	10/13/53	138	138	0	3.5	3.75	+0.25
42	10/14/53	142	144	0	3.75	3.5	—0.25
43	10/14/53	...	...	..	4.75	4.5	—0.25
44	10/15/53	142	144	+2	4.0	4.0	0
45	10/15/53	142	144	+2	3.25	3.25	0
46	10/15/53	132	134	+2	3.75	3.75	0
47	10/15/53	134	134	0	3.25	3.25	0
48	10/16/53	138	140	+2	4.25	4.25	0
49	10/16/53	140	142	+2	3.5	3.25	—0.25
50	10/16/53	150	148	—2	4.25	4.25	0
51	10/16/53	146	146	0	5.5	5.5	0
52	10/16/53	136	136	0	3.5	3.25	—0.25
53	10/17/53	146	148	+2	4.25	4.0	—0.25
54	10/20/53	144	144	0	4.25	4.25	0
55	10/21/53	140	138	—2	....	....	......
56	10/21/53	146	146	0	3.0	3.25	+0.25
57	10/21/53	134	132	—2	3.75	3.75	0
58	10/21/53	158	162	+4	....	....	......
59	10/23/53	144	144	0	....	....	......
60	10/23/53	136	136	0	....	....	......
61	10/26/53	140	140	0	3.0	2.75	—0.25
62	10/26/53	132	134	+2	5.25	5.0	—0.25
63	10/26/53	138	140	+2	3.5	3.25	—0.25
64	10/27/53	148	144	—4	3.5	3.75	+0.25
65	10/27/53	148	144	—4	4.0	3.75	—0.25
66	10/27/53	168	163	—5	....	....	......
67	10/27/53	...	...	..	3.5	3.75	+0.25

(cont'd)

		SODIUM TABLE			POTASSIUM TABLE		
No.	Date	Na.— mEq./L. Daily Curve	Na.— mEq./L. Master Curve	Difference	K— mEq./L. Daily Curve	K— mEq./L. Master Curve	Difference
68	10/28/53	148	144	—4	3.0	2.75	—0.25
69	10/28/53	140	140	0	4.5	4.5	0
70	10/28/53	142	140	—2	4.25	4.0	—0.25
71	10/28/53	150	148	—2	6.0	5.5	—0.5
72	10/28/53	144	144	0	....	....	......
73	10/28/53	144	144	0	....	....	......
74	10/30/53	134	134	0	2.25	2.5	+0.25
75	10/30/53	140	140	0	4.50	4.5	0
76	10/30/53	140	140	0	2.25	2.25	0
77	11/ 5/53	148	146	—2	....	....	......
78	11/ 5/53	142	144	+2	....	....	......
79	11/ 5/53	148	148	0	3.5	3.5	......
80	11/ 5/53	148	148	0	....	....	......
81	11/ 5/53	144	144	0	4.0	4.0	0
82	11/ 6/53	134	136	+2	3.5	3.5	0
83	11/ 6/53	134	136	+2	4.5	4.5	0
84	11/ 6/53	148	148	0	3.5	3.5	0
85	11/23/53	134	134	0	....	....	......
86	11/23/53	144	144	0	4.5	5.0	+0.5
87	11/23/53	140	140	0	3.75	4.5	+0.75
88	11/23/53	146	144	—2	2.75	3.0	+0.25
89	11/25/53	142	144	+2	....	....	......
90	11/25/53	146	148	+2	....	....	......
91	11/25/53	146	146	0	....	....	......
92	11/25/53	142	144	+2	....	....	......

Out of 89 Sodium determinations using both the Master Curve and a Daily Constructed Curve, 52 determinations were exactly the same, 23 gave a plus value and 14 a minus value. The mean deviation was +2.2 and -2.6 mEq. The highest one being +4 mEq. and —5 mEq. respectively.

just determined. Now add the patient's diluted serum into your funnel and turn your balance dial until the galvanometer needle reads zero. Record your balance dial reading. From either your master curve or else a calibration chart obtain your sodium value for that balance reading.

The potassium value may be determined in the same way, using your potassium filter, your normal potassium standard (0.1 mEq./liter) and your potassium master curve or calibration chart. Both of these values may be determined within five minutes time of operation.

Comparison Tables

I have compared 89 sodium tests and 68 potassium tests using the master curve technique and the daily constructed curve.

Out of 68 potassium determinations using both the master curve and a daily constructed curve 32 determinations gave the same value, 19 gave a plus value and 17 gave a negative value. The mean deviation was +.29 and —.26 mEq. The highest one being +0.75 and —0.5 mEq. respectively.

Summary

In conclusion this method seems quite accurate to use for "Stat" sodium and potassium when the technician's time is at a premium and would not permit the use of her time for 20-30 minutes on one determination alone. Also the simplicity of the method is akin to the reading of a blood sugar, etc., on the spectrophotometer. Because of the rapidity of the method and its accuracy, the physician in an extreme emergency may now be able to obtain satisfactory results on both sodium and potassium to enable him to maintain his patient in electrolyte balance.

It is understood that this method ought not to be used for routine sodiums and potassiums but only for emergencies. Also I have used this technique on the Janke Flame Photometer and do not know how it would correspond on other machines.

REFERENCES

1. Berry, J. W., Chappell, D. G. and Barnes, R. B. Ind. and Eng. Chem. Ed. **18** 19, 1946.
2. Fox, C. L. Jr., and Baer, H. Am. J. Phys. **151,** 155, 1947.
3. Lowry, O. H. and Hasting, A. B. J. Biol. Chem. **143** 257, 1942.

CONTINUING EDUCATION THROUGH SMALL HOSPITAL RESEARCH

JAMES D. BARGER, M.D.*

It would seem that the emphasis in research today is concentrated to an unhealthy extent in large cooperative programs with magnificent buildings, large staffs and ample money, and not enough encouragement is given the individual worker in the small institution. This should not be construed that such large programs are unnecessary, undesirable, or not productive of much fundamental knowledge, but let us not overlook the fact that the individual working along with simple tools can and will make contributions of significance. One of our leading scientists said "what is needed for research is a four-by-four room and a set of brains." Perhaps, this is over-simplification, but still there is much truth in his statement.

My experience has been that something has gone askew with almost every procedure in the clinical laboratory at some time. Much time is spent attempting to discover the cause of the trouble. These annoying events result in research, since the word means to search into or investigate carefully. Further, this is a contribution to the education of the individual.

In the busy work-a-day routine of the average hospital, little time is left for the pursuit of such things as a research program. In spite of this, an effort should be made to do some type of investigation, even of the most simple problems. Such a program

* Director of Laboratories, Good Samaritan Hospital, Phoenix, Arizona.

leads to better morale in the laboratory and makes for pride in one's profession.

Recently, medicine was depicted as no longer one of the "learned" professions but as the "learning" profession. This must also apply to those associated professions existing for the care of the patient. Education of the individual must be carried on consciously or unconsciously after the completion of the formal period of training if we are to serve the patient in the best manner. Preferably, there should be some sort of an organized program, the prime ingredient of which is the pathologist. He must be conversant and interested in all phases of clinical pathology, and have a desire for continued self-education. The technician must have interest, the will to learn, and a thorough knowledge of basic technics. With these basic necessities, it is not difficult to establish a program of continuing education.

In our hospital such a program is in the process of growing. Not all of the technicians participate nor is it expected they will, since participation is solely a result of the interest of the individual. Each is encouraged to pursue his particular interest. The necessary equipment is in the laboratory as are almost any reagents which may be needed. The laboratory library is well supplied with books and journals available to any member of the staff. If specimens from patients are required, these can usually be secured without difficulty through the cooperation of the clinicians.

One of the essentials of research is the communication of results. At first this was by informal discussions but with more interest in various problems, the need for a weekly or biweekly discussion conference became apparent. This serves to make all of the technicians in the department cognizant of what is being done and offers an opportunity for discussion and criticism.

The financing of such an endeavor is very important. In our laboratory, there is a special fund which is solely for educational purposes. This fund pays all subscriptions for the various journals, of which there are about fifteen. All books are purchased from this fund, as is any special equipment or reagents needed for special projects. These expenditures amount to $150.00 per month or less. This has been found adequate for all our needs.

Such a program in the small hospital is not one which can be born in full flower, but must grow like "Topsy," adapting itself to the needs of the situation. It is designed for self-education and improvement, and its prime objective is always better patient care. If this is the result of such a program, I feel that the expenditure of time, energy, and money is justified. Should we add something worthwhile to the general knowledge, then we are fulfilled beyond our hopes.

THE GAVEL

It has been said that educational experience has to be a continuing one. Educational experience in the broad sense implies a whole circle of the academic, cultural, social, and professional experiences which combine to make a rich and happy life. Our facet of this whole, professional education, is to be earnestly and diligently sought after, not only for personal edification but also for fulfillment of our individual obligations toward our professional development.

It is within the power and ability of each one of us to discover that daily work can be challenging and exciting. Clinical laboratory medicine is dynamic. New techniques are being developed; new concepts are being tested and proved; new knowledge is constantly being made available. It is all there for us to share—to be a part—if the effort is made to participate by constant study and active interest.

The advantage of additional formal class room study can be available in almost every community. Independent research on projects can be formulated and executed. The habit of reading at least one scientific journal faithfully can be cultivated. There is no stopping place in learning. Planning seminars, refresher courses, scientific programs, can be stimulating and valuable. There is much to be accomplished in the improvement of teaching programs and techniques. We can all do these things. They are open to all and are there waiting for those who have vision and enthusiasm to plan and the interest and the initiative to start.

In conclusion, be curious enough to investigate new methods and techniques. Be diligent enough to work accurately and inquiringly. Be earnest enough to study faithfully. Be enthusiastic enough to teach students truthfully. Be courageous enough to express ideas and opinions vocally and in writing. Be proud enough of your profession to contribute to its growth by active participation in constructive programs. You will be rewarded with personal satisfaction and happiness.

RUTH HOVDE

ABSTRACTS

BIOCHEMISTRY

AN IMPROVED METHOD for Multiple Rapid Determinations of Arterial Blood pH. Duncan A. Holaday (Columbia University, New York) J. Lab. Clin. Med. 44, 149-59 (1954).

An effective means of monitoring the pH of blood frequently and conveniently during anesthesia. The arrangement of the instrument permits direct transfer of blood from the patient into a glass electrode for the determination of pH at body temperature. Problems relating to obtaining the pH of biologic fluids and problems of the set-up of the apparatus are discussed.

BACTERIOLOGY

THE DIAGNOSIS OF BACTERIAL ENDOCARDITIS. Buford Hall (Lexington, Ky.) J. Kentucky State Med. Assoc. 52, 501-4 (1954).

A positive blood culture is the sine qua non of diagnosis. Six blood cultures taken at hourly intervals is suggested. Four patients revealed positive results on the second, third, and fifth cultures respectively. The remaining three cases had positive cultures on the initial venepunctures. Thus all positive cultures were obtained within the first five attempts and 90.5 per cent of these were positive on the first attempt.

Bacteria involved in forty-nine cases were the alpha streptococcus (viridans) in thirty, unknown in seven, enterococcus in five, staphylococcus in four, and gamma streptococci in three.

Positive blood cultures are less likely found when the infections are on the right side of the heart and the pulmonary artery than when the infections with vegetations are on the mitral or aortic valves.

Antibiotic therapy may be started after six blood cultures obtained at hourly intervals. Adjustments in dosage and choice of drugs may be made after identification and sensitivity tests of the organisms involved.

HEMATOLOGY

INCIDENCE OF HEMOGLOBIN C TRAIT in 505 Normal Negroes. A Family with Homozygous Hemoglobin C and Sickle-Cell Trait Union. Rose G. Schneider (University of Texas, Galveston) J. Lab. Clin. Med. 44, 133-44 (1954).

Filter-paper electrophoresis of the hemoglobin showed an incidence of 3.0 per cent of

hemoglobin C trait in an incidence of sickle-cell trait in 505 Negro blood bank donors.

Hemoglobin C ranged from 28-38 per cent in the hemoglobin samples from seven individuals with hemoglobin C trait. Hemoglobin C was 37 and 55 per cent respectively in two individuals with sickle-cell-hemoglobin C disease.

One family with homozygous hemoglobin C and sickle-cell trait union have one child with sickle-cell-hemoglobin C disease and the other two children have hemoglobin C trait.

The relationship of hemoglobin to the erythrocyte stroma is discussed. Also cited are that experiments are in progress concerning the production of a specific antiserum against sickle-cell anemia and whether rabbit antiserum against erythrocytes containing homozygous hemoglobin C can be formed.

STUDIES ON Rh. II. The Antepartum prediction of Hemolytic Disease in the Newborn with the Aid of Antihuman Globulin Serum. John S. Zelenik, Manuel D. Altamirano, and Harry Prystowsky (United States Army Hospital, Fort Benning, Ga.) Am. J. Obstet. Gynecol. 68, 633-8 (1954).

Observations obtained from a study March 1, 1952 through June 30, 1953 of sixty-five mothers whose blood demonstrated albumin or albumin and anti-human globulin antibodes:

1. When only albumin titers are present the infant will not be affected with hemolytic disease of the newborn and infants' Rh type cannot be predicted.

2. Infants of thirty-four mothers who developed anti-human globulin titers were Rh positive.

3. Where the anti-globulin titers were 1:8 or more, the Coombs' test on the infant was also positive; less than 1:8 resulted in a negative Coombs' test in the infant and a variable degree of disease in the infant.

4. When a sudden sharp rise occurred (4 or more tubes) in the antiglobulin titer in one or two week interval a moderately to severely affected infant resulted. The earlier this rise, the greater risk to the infant.

NEWER STANDARDS IN HEMOGLOBINOMETRY. Francis T. Flood, Emanuel E. Mandel, Raymond H. Owings, and Charles F. Federspiel. (Communicable Disease Center, Georgia). J. Lab. Clin. Med. 43, 897-904 (1954).

The method is based on the empirical finding that the ratio of optical densities of two independent color standards is reasonably constant for all photoelectric photometers of the same type. The ratio of the optical density of a standard solution of oxy-hemoglobin (blood containing 15 grams of hemoglobin per 100 ml. in suitable dilution) to the density of a standard cupric ammonium sulfate (Drabkin's) was found to be constant among six Klett-Summerson photometers. The owner of another Klett photometer may calibrate his instrument by preparing gravimetrically the standard copper solution, determine its optical density (D_{cu}) and multiply this value by the DR ratio characteristic of his make of instrument, viz., 0.984. The result is the optical density of the standard blood which permits calculation of the calibration factor (K) for the instrument:

$$K = \frac{\text{Concentration of standard}}{\text{Density of standard}} = \frac{15}{DR \times D_{cu}}$$

The hemoglobin concentration of any other blood sample can then be readily determined from its optical density reading ($D\,HbO_2$) by means of the usual equation:

$$Hb = D\,HbO_2 \times K$$

Investigation was made of the validity of gravimetrically prepared cupric ammonium sulfate solution and of a commercially supplied stable solution of reduced hemoglobin as color standards in hemoglobinometry. The respective ratios of densities (DR ratios) computed in accordance with Drabkin's principle with the copper standard and a blood specimen of known hemoglobin content were obtained with three types of photometers (19 instruments) with repeated optical density readings.

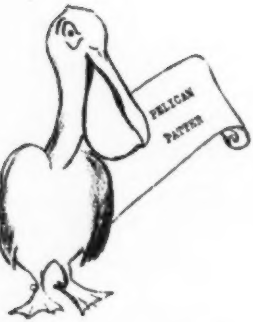
The DR ratios by six Klett-Summerson filter photometers fell within $\pm$ 1.2% of the mean DR value, while the corresponding ranges for ten Coleman Junior and three Coleman Universal spectrophotometers were $\pm$ 6.8 and 8.3% respectively. This better reproducibility with the Klett photometers is attributed to the greater width of the spectral band.

It is shown that hemog'obin equivalents rather than actual hemoglobin concentration of the standard is required for reliable photometric measurement of oyxhemoglobin by this means. The reliability of the method is enhanced by a considerable stability of the material if kept under proper refrigeration and anaerobic conditions.

PARASITOLOGY

NEW METHOD for Study of Intracellular Parasites with the Electron Microscope. B. N. Dutta, N. N. Das Gupta, M. L. De, A. Guha, and S. Nandi (Institute of Nuclear Physics, Calcutta). Science 120, 428-30 (1954).

The methods for overcoming the difficulty of such examinations are cited. Collodion-coated glass slides are smeared with the infected blood and the slides fixed by exposure to the vapor of 2 per cent OsO_4 solution for 3 minutes, carefully hydrolyzed in normal HCl at 60° C. for 10 minutes and then again washed in distilled water. When thoroughly dry, the collodion film with the hydrolyzed smear of blood is stripped from the glass and mounted on steel mesh for examination under the electron microscope. The various stages of the parasites are described. The hydrolysis of the fixed cells reduces the masking effect of the cytoplasm.



Well, we were getting a little restive worrying about whether our plans for next year's convention were progressing as well as they should so we sought out old Patate, the Pelican, who had so glibly last month promised to help us give you all one of the best times you've ever had.

We finally found him complacently sitting on one of the hawsers down at the river front, lazily watching sea gulls soar and dip and delicately pull little fish out of the river. The gulls and pigeons eyed him with interest because pelicans do not often visit so close to the City. In fact, his presence there made us think that he had been working.

"Well, Papa Patate," I said, "what have you planned for my friends as you promised?"

He winked one big eye and flapped a wing before him, "Don't have to plan—it's all here. You plan—programs, meetings, dinners, banquets, etc.—you tell them about that next time—the what you call them—the social affairs. I show them what is here all the time—the things they really come to see—the things that make La Nouvelle Orléans the fascinating city she is." I sit here and I look and I say to myself "Patate," I say, "we show them first Old Man River—because that mighty Mississippi just about makes N. O.—and a river trés intéressante she is! Aussi—just about the most important river in all the world. Nobody with any romance in his soul—your friends got romance?" he asked quickly, fearful—"no one with romance could come down and watch all these fine ships docking from every port of the world without his heart—she's lifted. Look over there—mahogany from South Africa—behind us—Coffee—six million bags every year! That next ship, crude rubber—and lá-bas—cotton, cotton going everywhere! And anybody who hasn't ever watched them unload bananas just is plain provincial! See all those blonde red cheeked boys over there—that ship she is from Rotterdam. Over there, that's a Chilean sailing vessel. c'est belle, n'est pas? If you do not keep your friends too tied up in meetings—they come down here, yes—they take a nice stroll along the wharves, maybe they ride the ferryboats—half-mile each way across—wonderful sights—cool—cheap—maybe they get on the big paddle wheeler and take a river excursion.

"Maybe your friends—even—they stay over after the convention and take a little cruise—to Havana or Guatemala or Jamaica or even Rio—might take advantage being so near. But, this river—she is—how you say?—commercial. Maybe the ladies they like more fun—Eh, bien! Tiens! We turn them right about and take them out to our lake—to our beaches, and sail boats and nearly six miles of sea wall and steps down to its water. Plenty playgrounds—plenty steps to stretch out on to get sunburned—plenty amusements—plenty crabs and shrimp and fish to eat!

"The ladies, too, they want to see the beautiful homes—where all these Creole beauties and Southern belles dish out all the mint julep hospitality. Well, we take them where all its first fine French and Spanish people live before Napoleon, he sell us off quick to the U. S. A.—that's down in the French Quarter and the Esplanade—interesting architecture—too sophisticated to be quaint—lovely courtyards—pretty patios—old world charm—then we take them up to the Garden District—Mes amis!—That's a place!—enormous, elegant, palatial homes like no where else—that's where the rich Americans lived when they first came down here right after Mr. Jefferson he bought us from Old Bony—you can't let your—how you call them—your technologists go home without they see that—then they go further uptown—around Audubon Park and the universities—they like to see them, too—and finally—ah, those slick new modern wonders out near the lake—you know, people who live in glass houses—very new—very streamy lined—very amusing!

"Oh, they gonna like N. O.—so much to see—so much to do—they know about Canal Street?—one of the widest streets in the world—used to be an old drainage canal to take the rain water off—now bright as Broadway—somebody said once—somebody important—there's no prettier sight in the world—Champs Elysées included—than Canal Street on an early summer evening just at twilight when the sky is still blue and the lights begin to come on—Oh, they gonna love N. O.

"Next time, I gonna tell you all about the Vieux Carré—the old French Quarter—Ah, mais c'est intéressante—ca! There isn't anyone coming to your convention who won't find something special—just for him—in the French Quarter—antiques—hundreds of shops—more shops, all together than anywhere in the country, night life—gayer than N. Y. or Montmartre and more of it!—Girlies and burlesques—and history and culture—but simply charmant—They gonna love N. O."

Au Revoir,
Patate le Pelican

FROM THE PROGRAM COMMITTEE

Patate has warned us that it is up to us to plan the scientific part of the convention. He has agreed to see to it that you will have the best time ever, but we must not lose sight of the fact that we are a scientific organization. So won't each and every one of you join effort with us in making this a truly outstanding convention? This can be accomplished by writing a paper or by preparing a scientific exhibit for the 1955 convention.

New Orleans is a medical center and already we have procured several outstanding program activities and scientific exhibits for you but—we want this to be an ASMT convention, which means your active participation.

Affiliate Society Presidents can encourage medical technologists in their state to write papers by sponsoring paper writing contests. Do you realize that if each affiliated society would send in one paper for the program, it would mean 50 papers?

Moreover, as ASMT members you are eligible for the Registry, ASMT, Hillkowitz and Scientific Products Awards. SO DO NOT DELAY—BEGIN NOW TO WRITE A PAPER OR PREPARE A SCIENTIFIC EXHIBIT!

IF YOU INTEND TO SUBMIT A PAPER FOR THE 1955 CONVENTION, WHETHER FOR AN AWARD OR NOT, YOU MUST NOTIFY THE PROGRAM CHAIRMAN BEFORE DECEMBER 15, 1954.

PAT SALLAS, Program Chairman
Department of Pathology
Charity Hospital of Louisiana
New Orleans, Louisiana

We wish to thank the State Presidents for responding so promptly to our request concerning the 1955 Convention Program.

AVAILABLE AWARDS

1. ASCP Registry Award of $50.00. All Registered Medical Technologists (ASCP) eligible. Work need not be original.
2. ASMT Awards of $100.00, $50.00, and $25.00. Only ASMT members are eligible. Work need not be original.
3. Parasitology Award of $25.00 for best paper on Parasitology. Only ASMT members eligible.
4. ASMT Awards of $75.00 for best State Society exhibit and $50.00 for best individual member exhibit.

At this time we do not know if the Hillkowitz Memorial Award will be available for the coming year.

PROGRAM RULES

1. The deadline date for the receipt of papers is *March 15*.
2. All competitive papers must be presented at the Convention in person or by proxy.
3. All papers submitted to the Society become ASMT property. They may be published in the American Journal of Medical Technology. Prize papers from State contests are eligible for awards if they have not been published.
4. Thirty minutes is the time allowed for each paper. Of this time, twenty minutes will be allowed for the reading of the paper and the remaining ten minutes will be for slide showing and discussion. Papers may be abstracted for the program and longer for publication.
5. Indicate on the fly sheet of the paper the following information: 1. The awards for which the paper is submitted; 2. Author's name; 3. Title of paper; 4. Whether or not work is original; 5. Whether or not article has been published.
6. Five copies of a typewritten, double spaced paper must be submitted

if the paper is to be considered for all awards.

7. Two copies of each manuscript must be submitted if not competing for the awards subject to the above instructions.

8. All audio and visual aids will be supplied by the Speakers' Supplies Committee. Please indicate what aids are needed for presentation of paper.

PROGRAM COMMITTEE

Patricia Sallas, Department of Pathology, Charity Hospital, New Orleans, Louisiana.
Genevieve Stout, 118 Forrest Boulevard, Decatur, Georgia.
Mrs. Jean Saumur, University of North Dakota, Grand Forks, North Dakota.
Bernice Koster, 3511 Park Avenue, St. Louis, Missouri.
James Parrott, Valley Baptist Hospital, Harlingen, Texas.
Mrs. Augusta Clark, Dept. of Pathology, Charity Hospital, New Orleans, La.

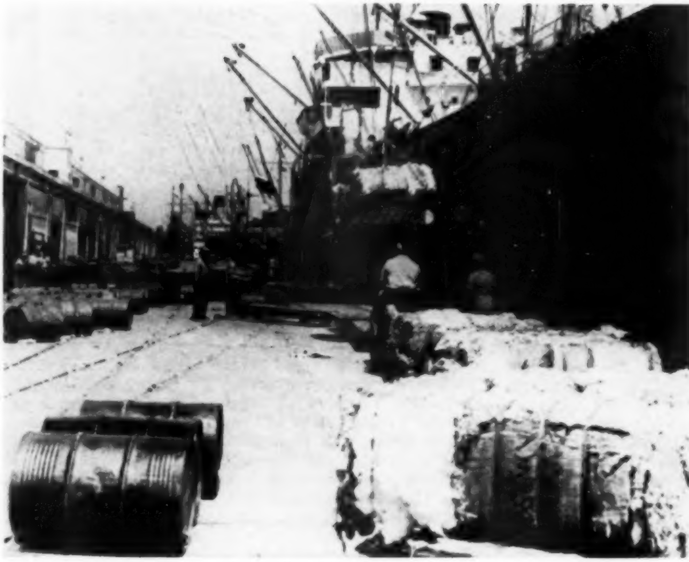
View of New Orleans Skyline looking from the River to the Lake

1. **CONVENTION HEADQUARTERS . . . Jung Hotel.**
2. **Steamer President. Entertainment Committee has planned a delightful trip on this boat.**
3. **Famous Canal Street which divided the City into two sections.**
4. **Uptown Section—Modern or New Section of City.**
5. **Downtown Section—Old Section of City containing the Vieux Carre and old world charm.**

FROM THE ENTERTAINMENT CHAIRMAN

Your entertainment committee, standing amid so much that has been storied and sung, has asked themselves where shall we start to tread the threshold of the wider world you may want. Will it be in the world famous restaurants with arpeggios of brandy set aflame; the world's widest thoroughfare (Canal Street); the Vieux Carre; the Universities; the Medical Center; Lake Ponchartrain; the shops where the routes of commerce deposit their riches for the treasure hunter, or will it be where the valley's riches come to meet the sea?

Since the days of sail and stagecoach, pirates and swords, the world famous romantic harbor at the Mississippi's busiest bend has given shelter and refuge to the men of the seven seas. So—Patate suggested that we arrange for you a 2½ hour trip on the mighty Mississippi at N. O. aboard the largest sight-seeing Steamer "President" to give you a complete picture of the place the Crescent City occupies as a commercial center in



Loading Cotton on Wharf

the U. S. and give you a chance to see Cole Porter's inspiration for the magnificent production "Showboat."

Just think of five spacious decks where you can rest, relax and dream of the crinoline days in solid comfort, while more than one hundred thrilling, interesting and unforgettable sights are explained by an experienced Guide. Here you'll see ocean vessels with the breeze fanning their exotic flags, luxurious liners and freighters from all parts of the world, more than 13 miles of wharves—the famous coffee, banana, and cotton docks—largest in the world, giant grain elevators and side ship warehouses. You will see the $20,000,000 Industrial Canal, Intercoastal Harvey Canal, gigantic Huey P. Long Bridge, $15,000,000 Army Supply Base, U. S. Immigration Station, huge Floating Dry Docks, Chalmette Sugar Refinery, Public Commodity Warehouse, U.S.P.H.S. Hospital and many other sights that will give you a sense of growing close to much you used to feel was far and foreign. Annual exports and imports handled through this port are valued at 1½ billion dollars. More than 3,300 ocean going vessels enter the port each year and water borne commerce totals 30 million tons. It will be an afternoon spiced with excitement and enjoyment and when your Steamer docks at the foot of Canal Street at 5 P.M. it will be a reluctant au revoir to "OLD MAN RIVER" but there are many surprises awaiting you so, you too must go rolling along for this is no "Make Believe" but a reality in a City where the blueprint and the Budget include consideration for the soul.

Watch your ASMT Journals for the activities of your entertainment committee for we have so much more to tell you. Don't forget it's time to start saving for June 12 to 17th, 1955 and remember it's a date.

FROM THE EXHIBIT COMMITTEE

It is not too early for you to reserve space for your scientific exhibit. Fellow technologists are always eager to see and learn new ideas and accomplishments.

For particulars concerning the exhibit, write

HELEN BEROS,
Scientific Exhibit Chairman
4234 St. Claude Avenue,
New Orleans 17, La.

COURSE IN EXFOLIATIVE CYTOLOGY

A course in Exfoliative Cytology will be given at the Papanicolaou Cytology Laboratory, Cornell University Medical College, March 7 to June 3, 1955. The program includes lectures, demonstrations, discussions, practice in the preparation and staining of smears, and practical instruction in the interpretation of exfoliative cytologic material. A certificate is given to those who attend the complete course and demonstrate progress satisfactory to the laboratory staff. Instruction will be given by Dr. George N. Papanicolaou. Dr. N. Chandler Foot, Dr. John F. Seybolt, Miss Charlotte M. Street and other members of the staff.

The course is open to both physicians and technologists. Requirements for the latter include: (1) Employment or contract for future employment by an approved hospital or laboratory, with supervision of the cytologic work by an adequately trained cytologist or cyto-pathologist. (2) A course in histology, including microscopic study of tissues as well as technic. Other related courses such as hematology, gross anatomy and pathology, though not required, constitute a valuable background.

Tuition for the course is $300.00.

Inquiries from technicians should be addressed to Miss Charlotte M. Street, and from physicians to Dr. John F. Seybolt, Papanicolaou Cytology Laboratory, Cornell University Medical College, 1300 York Avenue, New York 21, N. Y.

POSTGRADUATE COURSE IN MEDICAL TECHNOLOGY

Place:—University of Colorado Medical Center,
4200 East 9th Avenue, Denver, Colorado.

Date:—March 16-19, 1955.

Registration Fee:—$12.50 for entire course
$ 6.00 for single day

Principal Guest Speakers:—

Thomas Hale Ham, M.D., Western Reserve University School of Medicine, Cleveland Ohio.

Mervin H. Grossman, M.D., Director of Laboratories, St. Paul's Hospital, Dallas, Texas.

Sponsors:—University of Colorado Medical Center and Colorado State Society of Medical Technologists.

Entertainment:—Information will be provided on winter sports and tours.

Open to all professional personnel.

For Further Information and Application Blanks:— Contact the OFFICE OF GRADUATE AND POSTGRADUATE MEDICAL EDUCATION, 4200 East 9th Ave., Denver 20, Colorado.

ANNUAL POSTGRADUATE COURSE IN MEDICAL TECHNOLOGY

The University of Kansas School of Medicine announces its *Sixth Annual Postgraduate Course in MEDICAL TECHNOLOGY*, JANUARY 10, 11 & 12, 1955, to be presented at K. U. Medical Center, Kansas City, Kansas.

The course will deal with subjects in Hematology, Bacteriology, Mycology, Serology, Chemistry and miscellaneous laboratory procedures. In addition to didactic lectures, the program will be highlighted by demon-

strations, a discussion of the selection and care of laboratory glassware, films on "Phase Microscopy" and "The Normal Kidney," and a symposium as the closing feature of each day's program.

The distinguished guest faculty includes:

NORMAN F. CONANT, Ph.D., Professor of Mycology and Associate Professor of Bacteriology, Duke University School of Medicine, Durham, N. C.

EUGENE HILDEBRAND, M.D., Pathologist, Mercy Hospital, Denver.

J. N. McCONNELL, Manager, Chicago Division, American Hospital Supply Corporation, Evanston, Ill.

FRANKLIN R. MILLER, M.D., Hematologist, The Snyder Clinic, Winfield, Kans.

The course is open to all serving in medical laboratories upon payment of the $12.00 enrollment fee. For program announcement, write: Extension Program in Medicine, University of Kansas Medical Center, Kansas City 12, Kansas.

FROM OUR READERS

QUESTION from A.T.B., Houston, Texas: "Why do we record Sed. Rates every 15 minutes. When it takes a lot of concentration and they treat the patient the same as if they would if we only gave them the 1 hour reading?"

ANSWER: You might miss a case of myelomatosis with its hyperglobulinemia and rapid fall in the first 15 minutes of the sedimentation rate. Quoting from Wintrobe's CLINICAL HEMATOLOGY: "When frequent readings are made, the results may be plotted graphically. By this means every variation from an almost horizontal line to one which falls quickly in a vertical direction may be encountered. Cutler demonstrated by means of such curves that the process of sedimentation consists of three phases; namely, a preliminary period during which aggregation of the red corpuscles takes place, (2) a period of rapid fall and (3) the phase of packing when the corpuscle masses, in accumulating at the bottom of the tube, slow up the rate of sedimentation.

If a record is made of the erythrocyte sedimentation at frequent intervals, it is possible to measure the phase of rapid sedimentation which is, of course, the significant phase. When readings are made only at the end of one hour, the figure obtained is influenced by packing of the corpuscles."

QUESTION from A.T.B., Houston, Texas: "Why does the physician want to know the color and reaction of the urine? Is it just because the request slip is printed that way or do they get something out of it?"

ANSWER: The medical technologists might learn more on these questions by reading what medical students are taught. Joel T. Shrager, M.D., ASCP, Clinical Pathologist at Veterans Administration Hospital, Dearborn, Michigan, teaches urinalysis to the sophomore medical students at Wayne University—College of Medicine. Quoting from his lecture notes: "The color of urine is normally yellow or amber due to the presence of pigments such as urochrome, urobilin, uro-erythrin and porphyrins. Dilute urine of low specific gravity, as occurs in polyuria, is usually pale yellow or greenish yellow, while scanty, concentrated urine, as in oliguria, is usually dark amber. Acid urine is generally darker than alkaline urine. The color of urine may be greatly changed by the presence of blood, melanin and other abnormal pigments, or by various drugs and poisons.

Erythrocytes produce a smoky-red to brownish-red colored urine, hemoglobin a red to reddish-black, or black, hematoporphyrin and other porphyrins a port wine color, bilirubin and biliverdin a yellowish-green, urobilin—in excess—a dark brown, hemogentisic acid and other alkaptons a brown changing to black color, hydroquinone or pyrocatechin a smoky

dark brown or black, chyle or marked contamination with petrolatum or milk a milky-colored urine.

"Drugs taken by mouth but excreted through the kidneys will color the urine. Methylene blue or thymol will produce a greenish-blue, acriflavin a yellowish-green, santonin a golden yellow, pyridium an orange-red and neo-prontyril a dark red color.

"The acidity of urine is normally influenced by diet, fluid intake and also by various drugs. Cereals, meat and fresh fish tend to increase the acidity of urine, while most fruits, except plums, prunes and cranberries, tend to reduce it because of basic radicals (alkaline ash) and their rejection by the tubules. A high protein diet increases the acidity of urine because of the production of an excess of sulphuric and phosphoric acids which are eliminated by tubular rejection. Acidity is also increased by a diet sufficiently high in fats to produce ketosis, and also by an acid ash diet. Fasting and starvation in which body proteins are metabolized also tend to increase the titrable acidity.

"The administration of large amounts of hydrochloric, phosphoric and mandelic acids, as well as ammonium chloride, ammonium nitrate, ammonium mandelate and calcium chloride, tend to increase the acidity of urine. The maximum acidity attainable, however, is about pH 4.6.

"Alkaline urines are frequently observed in cystitis and pyelonephritis because of the formation of bacteria of ammonia from urea. Unless properly preserved, urine on standing ultimately becomes alkaline because of the formation of ammonia by contaminating bacteria."

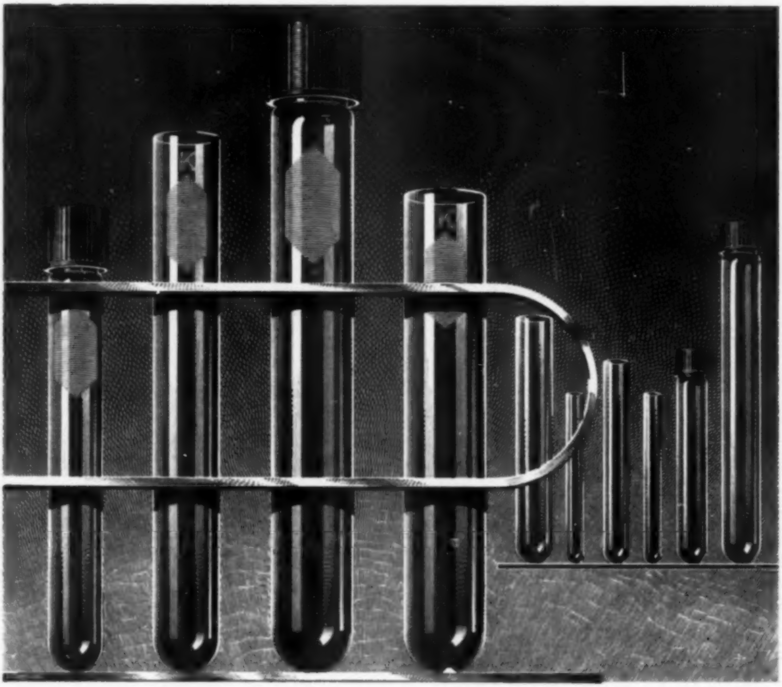
BACTERIOLOGICAL NOTES submitted by R. J. W., Reading, Pennsylvania.

The Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, reports that many infected wounds demand rapid, accurate anaerobic culture procedures. Since these tissues frequently contain relatively few organisms, conventional anaerobic culture methods often prove inadequate. In addition, the spreading growth of facultative anaerobes obscure Clostridia colonies, which can be recovered, if at all, only by time-consuming multiple platings. A combination of inhibitory substances was developed which includes the following in blood agar yeast extract plates:— chloral hydrate, 0.1%; sodium azide, 0.2%; sorbic acid, 0.15%, and Polymixin B, 0.001%. This group of substances inhibited a high percentage of the genera Proteus, Pseudomonas, Bacillus, and Staphylococcus, and provide considerable assistance in isolation of Clostridia.

The Department of Infectious Diseases, School of Medicine, Univ. of California at Los Angeles reports that although Brucellae are said to be non-fermentative, the utilization of glucose by Brucella abortus has been demonstrated in both manometric and growth studies. It, therefore, appears possible that qualitative carbohydrate fermentation tests might serve to distinguish species in the genus Brucella. A fermentation base has been developed which is applicable to tests of this type, and tried with 27 carbohydrates. Five carbohydrates were differentially fermented by 25 strains of B. abortus, 13 strains of B. melitensis, and 15 strains of B. suis. The positive tests with these five were as follows:—B. abortus—inositol, mannose, and rhammose; B. suis—maltose, mannose, and trehalose.

Kennedy Hospital, Memphis, Tenn., states that in previous investigations it was found that a modified human blood medium containing penicillin was satisfactory for the cultivation of tubercle bacilli under routine diagnostic conditions. Although this antibiotic is effective in reducing contamination in concentrates, a certain amount still results despite all precautions. It was found that 100 units of penicillin per ml. in combination with 0.01 percent malachite green appears to fulfill the need, and does not affect the rate and degree of tubercle bacilli growth or their colony morphology. Lowenstein-Jensen medium may also be used, but the contamination rate is higher than with the blood medium. The average time of the first appearance of growth was 21.3 days on the former medium, and 18.2 days on the latter medium.

A simple device for determining time and temperature of sterilization in the autoclave or hot air oven is suggested by the Dept. of Biological Research, Hynson, Westcott & Dunning, Inc., Baltimore, Md. Since one often is concerned with whether or not the sterilization has been properly carried out, a number of indicators have been marketed. Many of these show only that the temperature has been reached. Chemical melting points have always been acceptable as reliable indicators of temperature if they remain pure. By incorporating a proper compound in a sealed glass tube which permits the flow of the molten material, one may determine the temperature as well as the length of time the temperature remained constant. This apparatus is small, inexpensive, and may be used countless times. It also has the advantage that it can be included in packages and containers of materials for sterilization.



Kimble Culture Tubes are available with three types of caps: (1) cemented in pulp-backed vinylite liner; (2) cemented in rubber liner; (3) pressure fitted Teflon liner.

DURABLE!

KIMBLE CULTURE TUBES

Their long, useful life means low costs for you

Kimble Culture Tubes are made of Kimble N51A glass—a borosilicate glass with outstanding resistance to chemical attack.

They do not affect the growth or viability of cultures. They resist the chemical attack of washing solutions. Kimble Culture Tubes resist alkaline, acid and nearly neutral solutions, including hot water and steam.

Tubes easily withstand heat-shock of sterilization . . . are thoroughly annealed. Walls are of uniform thickness . . . bottoms are of ample weight to avoid "breaking-out."

New style, longer caps, when slightly loosened, permit free exchange between the atmospheres inside and outside the tubes, completely satisfying the gas requirements of aerobic cultures. Threads are so designed that there is no danger of the slightly loosened caps falling off.

See your laboratory supply dealer for information about Culture Tubes and the complete Kimble glassware line. Or write Kimble Glass Company, subsidiary of Owens-Illinois, Box 1035, Toledo 1, Ohio.

KIMBLE LABORATORY GLASSWARE
AN (I) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES · TOLEDO 1, OHIO

APPROVED

Cardiolipin-Lecithin Antigens

"A sample from this lot* has been tested in parallel with standard antigen by the Venereal Disease Research Laboratory and found to be of standard reactivity."

APPROVED . . .
Dr. John A. Kolmer

APPROVED . . .
Mazzini Sero-diagnostic Laboratory

APPROVED . . .
Dr. William A. Hinton

APPROVED . . .
Dr. C. R. Rein — H. N. Bossak

Specify

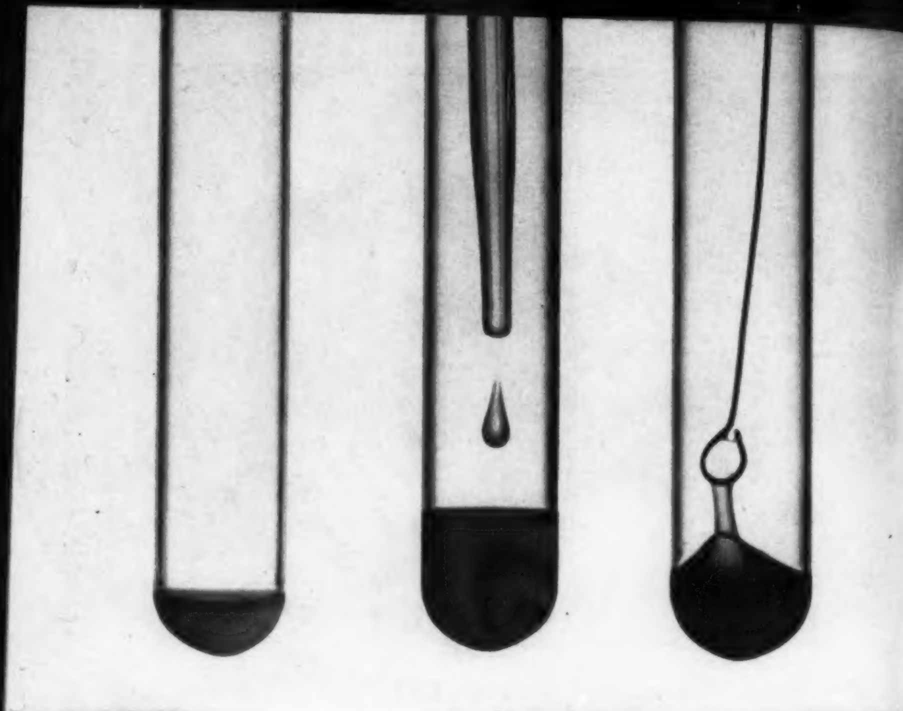
Sylvana

approved antigens

SYLVANA CHEMICAL COMPANY
ORANGE, NEW JERSEY

SYCCO

* *Each lot of SYLVANA cardiolipin-lecithin antigens is approved by the author-serologist or reference laboratory involved. Cardiolipin is produced under license from the New York State Department of Health.*



Act III—the clot thickens

In the drama unfolding daily in the technician's test tube, Simplastin plays a key role. Whenever it is necessary either to produce or to combat lowered blood prothrombin, Simplastin supplies the essential prothrombin time data . . . swiftly and accurately. Bio-assayed on normal human whole and dilute plasma, it assures sensitive reproducible end-points.

Simplastin fulfills the strictest requirements for a thromboplastin reagent. Preadjusted to optimum sodium chloride and calcium chloride content, Simplastin is instantly available for use merely with the addition of distilled water. Costly and time-consuming extra preparation steps are eliminated.[1]

In the laboratory, Simplastin saves valuable time and effort . . . in therapy, it assures more accurate and dependable results.[2,3]

Supplied in 2 sizes

Efficient 6-Determination Size for emergency use and for the smaller laboratory

Economical 20-Determination Size for the larger laboratory

Both sizes are supplied in boxes containing 10 vials. A free trial supply will be sent promptly upon request. . . . Available at all leading laboratory supply houses; write for the name of your local supplier.

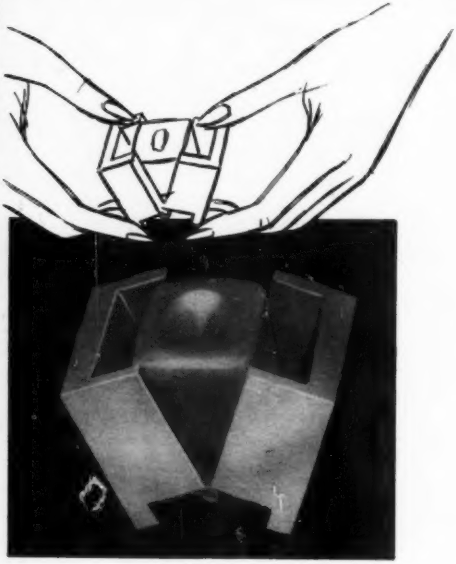
Bibliography:

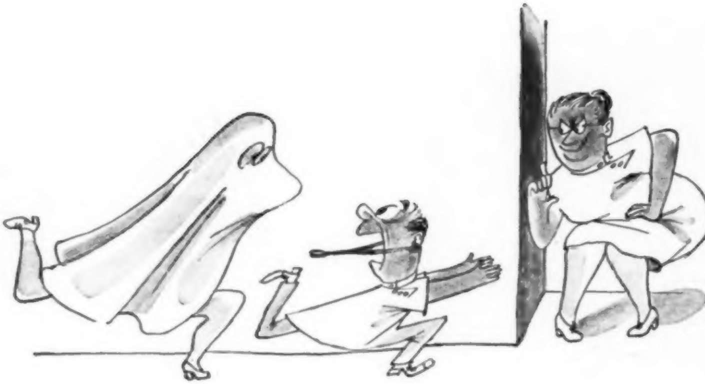
1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951.
2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

Simplastin® *simple* for the technician
accurate for the clinician

WARNER-CHILCOTT

VDRL Flocculation Antigen
For use in VDRL Slide or Tube Tests for diagnosis of Syphilis
Scientific Products Division
Buffered Saline Diluent
Cardiolipin Antigen for Kolmer Complement-Fixation Tests for Syphilis


O



OPPORTUNITIES

Chief Medical Technologist: Southwest. Private laboratory. Registered or equivalent in experience. Complete charge of active laboratory. Will participate on percentage basis after one year. $500 to start. ASMT-5952

Chief Medical Technologist: East. 100 bed hospital. Must have good administrative ability plus complete knowledge of all phases of laboratory procedure. ASCP registration is essential. Will act as assistant to pathologist who is certified. This is a real opportunity. Salary to $7000 to start. ASMT-6015.

Medical Technologist: 100 bed hospital located in scenic southwest. New laboratory—completely equipped with all modern facilities. $300 plus complete maintenance. ASMT-6147

Chief Medical Technologist: Southeast. 150 bed hospital, fully approved. ASCP registration plus good supervisory experience. $500. ASMT-5775

Medical Technologist: East. 230 bed hospital, fully approved. Located in city of 85,000. Must have at least 4 years experience. 14 technologists, 5 aides in laboratory. $475. ASMT-5394

Medical Technologist: South. Prefer some knowledge of X-Ray. 100 bed hospital. Good housing facilities. $400. ASMT-5630

Medical Technologist: South. Large hospital affiliated with a university; if desired can further education at no cost. Maintenance very reasonable. $350-$400. ASMT-6005

Medical Technologist: West. Blood bank experience. 4 technologists in department. City of about 90,000. Adequate housing reasonable. $350. ASMT-5898

Medical Technologist: East. 260 bed hospital—some supervisory work, teaching research and routine procedures. ASMT-5782

Chief Tissue Technician: East. 250 bed hospital. Complete supervision of tissue laboratory. $350. ASMT-5784

Medical Technologist: Southeast. 250 bed hospital in winter resort city. Require knowledge of chemistry. 5 in department. $350. ASMT-5473.

Medical Technologist: Middle West. Department of Medicine of well known University. Masters degree in Biochemistry or Bachelors in Chemistry. ASMT-5932

Medical Technologist: Middle West. University School of Medicine. Duties: prepare culture media and reagents—care for cultures, etc. 6 in department. B.S. degree in microbiology, biology or chemistry. $375. ASMT-5923

Medical Technologist—Blood Bank: Middle West. 350 bed hospital, fully approved. Complete charge of blood bank with two well qualified assistants. ASCP or good experience in blood bank procedures. $450. ASMT-6138

Medical Technologist: Middle West. 200 bed hospital. Some teaching—approved school for Medical Technologists. $400 minimum depending upon qualifications. ASMT-5761

Medical Technologist: Middle West. Experience in hematology and chemistry; also routine procedures. 20 bed hospital, fully approved. $350. ASMT-5843.

Chief Medical Technologist: South. 125 bed hospital. Qualified to teach in a professional school for medical technologists. $400-$450. ASMT-5116.

Medical Technologist: Southwest. 150 bed hospital, fully approved. Full time certified pathologist. $400. ASMT-6075.

Many other attractive opportunities in all parts of country. Write us for full particulars about our highly personalized service. Strictly confidential.

SHAY MEDICAL AGENCY

55 EAST Washington Street | **BLANCHE L. SHAY, Director** | **Chicago 2, Ill.**

HAVE YOU CHANGED YOUR ADDRESS LATELY?

PLEASE SEND US YOUR ADDRESS CHANGE so that you will receive your journal regularly. If you are going on a vacation "between journals" do NOT notify us. An address change authorized by YOU is the most satisfactory. Failure to let us know where you are located may be the reason you miss an occasional issue of the journal.

Name: ______________________

OLD ADDRESS ______________________

____

CHANGE TO: ______________________

____

Tear off and mail to

THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY
Suite 25, Hermann Professional Bldg.
Houston 25, Texas

INDEX TO VOLUME TWENTY

M

N

O

P

R

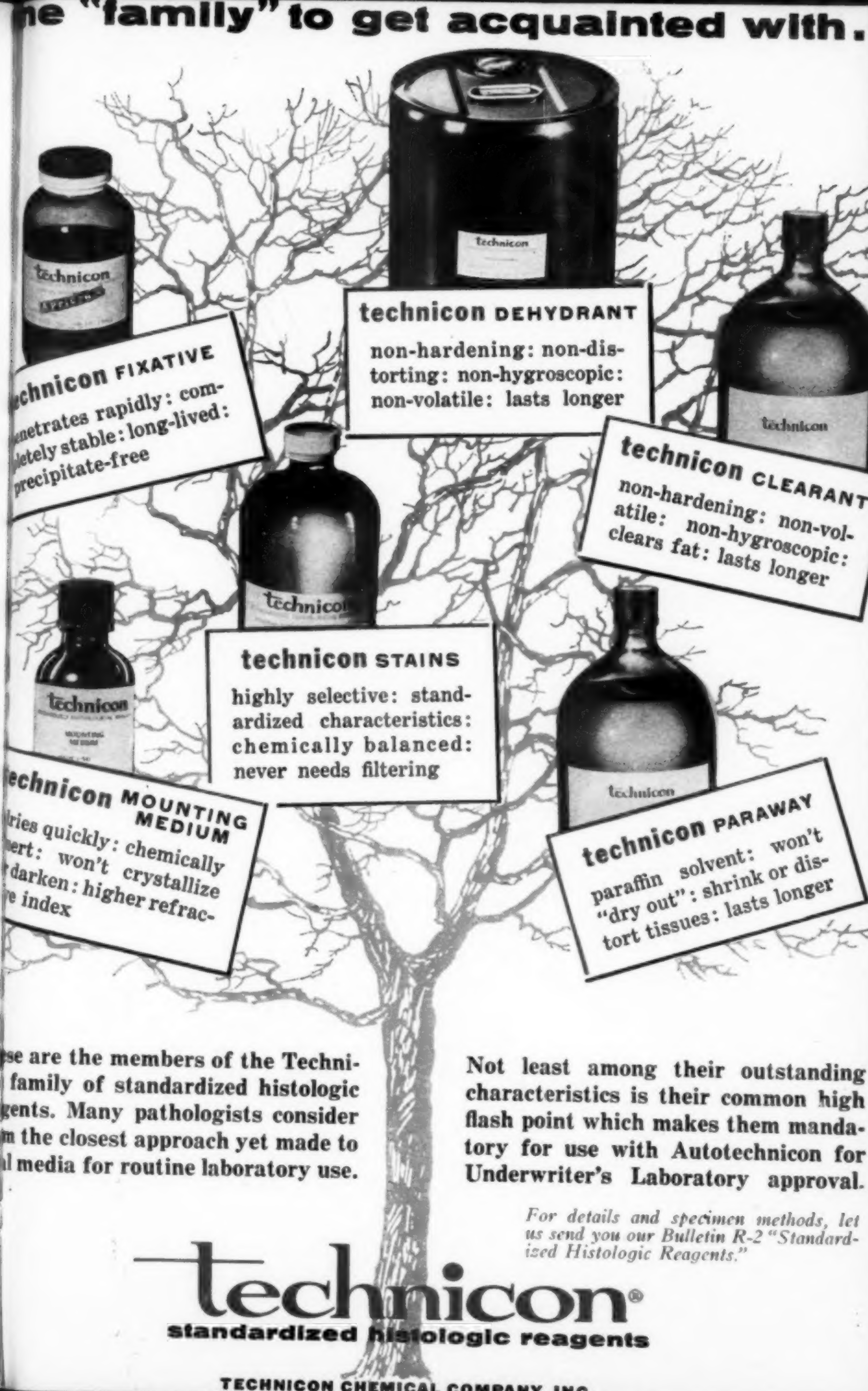
S

T

V

W

Z



ne "family" to get acquainted with.
technicon
technicon DEHYDRANT
non-hardening: non-distorting: non-hygroscopic: non-volatile: lasts longer
technicon FIXATIVE
enetrates rapidly: comletely stable: long-lived: precipitate-free
technicon
technicon CLEARANT
non-hardening: non-volatile: non-hygroscopic: clears fat: lasts longer
technicon
technicon STAINS
highly selective: standardized characteristics: chemically balanced: never needs filtering
technicon
echnicon MOUNTING MEDIUM
lries quickly: chemically ert: won't crystallize darken: higher refrace index
technicon
technicon PARAWAY
paraffin solvent: won't "dry out": shrink or distort tissues: lasts longer
se are the members of the Techni family of standardized histologic gents. Many pathologists consider m the closest approach yet made to l media for routine laboratory use.
Not least among their outstanding characteristics is their common high flash point which makes them mandatory for use with Autotechnicon for Underwriter's Laboratory approval.
For details and specimen methods, let us send you our Bulletin R-2 "Standardized Histologic Reagents."
technicon®
standardized histologic reagents
TECHNICON CHEMICAL COMPANY, INC.